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REVIEW

Role of T cell death in maintaining immune tolerance during persistent viral hepatitis

Juan Ramón Larrubia, Megha Uttam Lokhande, Silvia García-Garzón, Joaquín Miquel, Dolores Subirá, Eduardo Sanz-de-Villalobos

Juan Ramón Larrubia, Megha Uttam Lokhande, Silvia García-Garzón, Joaquín Miquel, Dolores Subirá, Eduardo Sanz-de-Villalobos, Translational Hepatology Unit, Guadalajara University Hospital, University of Alcalá, 19002 Guadalajara, Spain

Author contributions: Larrubia JR and Lokhande MU contributed equally towards the conception and design of the review; Larrubia JR and Lokhande MU co-wrote and Larrubia JR revised the manuscript; García-Garzón S, Miquel J, Subirá D and Sanz-de-Villalobos E contributed equally to the supportive work and supervision.

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Correspondence to: Megha Uttam Lokhande, MSc, Translational Hepatology Unit. Guadalajara University Hospital. University of Alcalá. Donante de Sangre st., 19002 Guadalajara, Spain. lokhandemu@gmail.com
Telephone: +34-949-209200 Fax: +34-949-209259

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Abstract

Virus-specific T cells play an important role in the resolution of hepatic infection. However, during chronic hepatitis infection these cells lack their effector functions and fail to control the virus. Hepatitis B virus and hepatitis C virus have developed several mechanisms to generate immune tolerance. One of these strategies is the depletion of virus-specific T cells by apoptosis. The immunotolerogenic liver has unique property to retain and activate naïve T cell to avoid the over reactivation of immune response against antigens which is exploited by hepatotropic viruses to persist. The deletion of the virus-specific T cells occurs by intrinsic (passive) apoptotic mechanism. The pro-apoptotic molecule Bcl-2 interacting mediator (Bim) has attracted increasing attention as a pivotal involvement in apoptosis, as a regulator of tissue homeostasis and an enhancer for the viral persistence. Here, we reviewed our current knowledge on the evidence showing critical role of Bim in viral-specific T cell death by apoptotic pathways and helps in the immune tolerance.

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Key words: T cell death; Specific cytotoxic T lymphocytes; Hepatitis C virus immune tolerance; Apoptosis; Bcl-2 interacting mediator; Liver tolerance; Apoptotic pathways; Viral hepatitis

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INTRODUCTION

Hepatotropic, non-cytopathic viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV) behave as intracellular parasites. The activation of cellular immune response by priming of naïve specific CD4+ and CD8+ T cells in the lymph nodes is very important to control viral infection. However, the unique ability of the liver to retain and activate naïve CD8+ T cells leads to liver tolerance, by-passing normal activation in the lymph nodes. The continuous triggering of antigen presenting cells (APCs) in the sinusoids by the antigen-rich blood leads to peripheral tolerance to protect the liver tissue. This physiological feature can be used by hepatotropic viruses as a persistence mechanism. The depletion of liver activated CD8+ T cells is the critical part of the peripheral tolerance in HBV/HCV infection. The anticipated mecha-

nisms for immune tolerance in liver specific pathogens are linked to virus-specific T cells death. The vital role of pro-apoptotic molecule, Bcl-2 interacting mediator (Bim) in the death of the virus-specific T cells has been shown after intrahepatic T cell activation by hepatocytes[1], in chronic HBV and HCV infection[2,3]. Therefore, this review provides glimpse of the recent advances to understand the cellular and molecular mechanism involved on "T cell death" during viral hepatitis as a viral escape mechanism through the induction of a specific-immunotolerant status on the host.

VIRAL HEPATITIS

HBV and HCV viruses are two hepatotropic noncytopathic, human blood-born viruses. HBV is a small, enveloped DNA virus that undergoes a pro-viral state to persist in the host. HCV is an enveloped virus with a plus-strand RNA genome. It has been estimated that more than 350 million for HBV and 170 million people for HCV are infected. Approximately 80% of infections in HCV and > 90% of infected neonates, 5%-10% of infected adults in HBV succeed in establishing a chronic infection, with the potential for developing severe liver diseases such as cirrhosis and hepatocellular carcinoma[4,5].

Highly productive and replicative viruses such as HBV and HCV are associated with ineffective antiviral immunity during persistent viral infections. The complex ineffective immunity involves the functional deterioration of antiviral T cell responses and contraction of the size of this response. In persistent HBV/HCV infections, T cells are continuously challenged by high levels of viral antigens that eventually result in limiting the antiviral T cell response and ultimately leading to T cell exhaustion. This is a progressive process, starting with the deficiency in cytokine production, proliferation and survival[6], to end with physical deletion of specific antiviral T-cell populations[7].

Meticulously, cytotoxic T lymphocytes (CTLs) play a vital role in viral eradication[8] and in the pathogenesis of hepatitis[9-11]. A strong, multi-specific and long-lasting T-cell immune response emerges to be important for control of viral infection[12-14]. Appropriate, polyclonal, vigorous and multi-specific CTL responses can facilitate complete viral clearance, in which long-lasting protective T cell response is observed. However, specific CTL responses are usually not strong enough to eradicate the virus, hence contributing to persistent infection[15,16].

HBV and HCV are hepatotropic viruses that replicate in the liver. This organ features a unique immune tissue, where the deletion of antiviral T cell populations has been shown, being involved in local and systemic immune tolerance.

LIVER AS A FOUNDATION OF IMMUNE TOLERANCE

Liver situates at a hemodynamic convergence, receiving the splanchnic stream, which means an intense contact with exogenous antigens. This fact leads to the development of tolerance mechanisms to avoid inappropriate immune system activation, but it also allows antigen presentation by resident cells. Therefore, the liver is progressively more being recognized as an immune organ[17]. Liver sinusoids, hepatic arteries and portal venous carry blood containing digested nutrients and micro antigens from intestine, and as a primary metabolic organ, the liver produces multiple neo-antigens. All these molecules pass through sinusoids and finally are taken up and metabolized by different hepatic resident cells. The liver has acquired specialized mechanisms of immune tolerance to avoid the over reactivation of immune response against antigens that are metabolized in the liver. In fact, this process may be beneficial for inducing tolerance to liver grafts but also to the liver specific pathogens. Therefore, hepatotropic viruses exploit these immunotolerogenic liver features to persist. It is important to remind that the liver has the ability to retain and activate naïve CD8+ T cells ineffectively, in contrast to other lymphoid tissues. This fact may allow pathogens to escape from T cell mediated immunity and establish a persistent hepatic infection due to immune tolerance induction. This immunotolerant state can be reached by the development of T cell anergy but also by specific T cell deletion.

Uniqueness of the liver

The unique character of the hepatic tissue to tolerate liver allograft across major histocompatibility complex (MHC) mismatch in the pig without immunosupression was described by first time in 1969[17]. Later studies confirmed that this occurred because of the induction of immunological tolerance in the liver[18]. Initially, "graveyard theory" suggested that the exclusive ability of the liver to get rid of activated T cells, programmed to undergo apoptosis, was the root of the hepatic tolerance effect[19]. This theory proposed two functions of the liver as a T cell graveyard: (1) passive killer of the liver cells after their life cycle; and (2) efficient killer of the activated antigen specific T cells. According to this theory, T cell receptor (TCR) triggering by cognate antigen on TCR transgenic T cells leads to activation and accumulation of those cells in the liver and undergoes depletion of mature T cells[20].

The theory was again proved by Mehal *et al*[21] by indicating that the normal liver is a "sink" for activated T cells. The liver was perfused by T cells showing retention of activated, but neither resting nor apoptotic T cells[21]. Liver as a graveyard for activated T cells theory forced to believe that all the immune response in the liver would be silent; in spite of this, the presence of an effective virus specific T cells in patients controlling hepatic viral infections[22,23] could challenge this theory. Nonetheless, the removal of activated T cells by the liver cannot be excluded, as evidenced by the ability of liver allograft to rescue rejecting skin grafts[21], in which lately tolerising capacity of the liver for activated allo-specific T cells occurs. In some cases, the limited capacity of the liver to induce tolerance

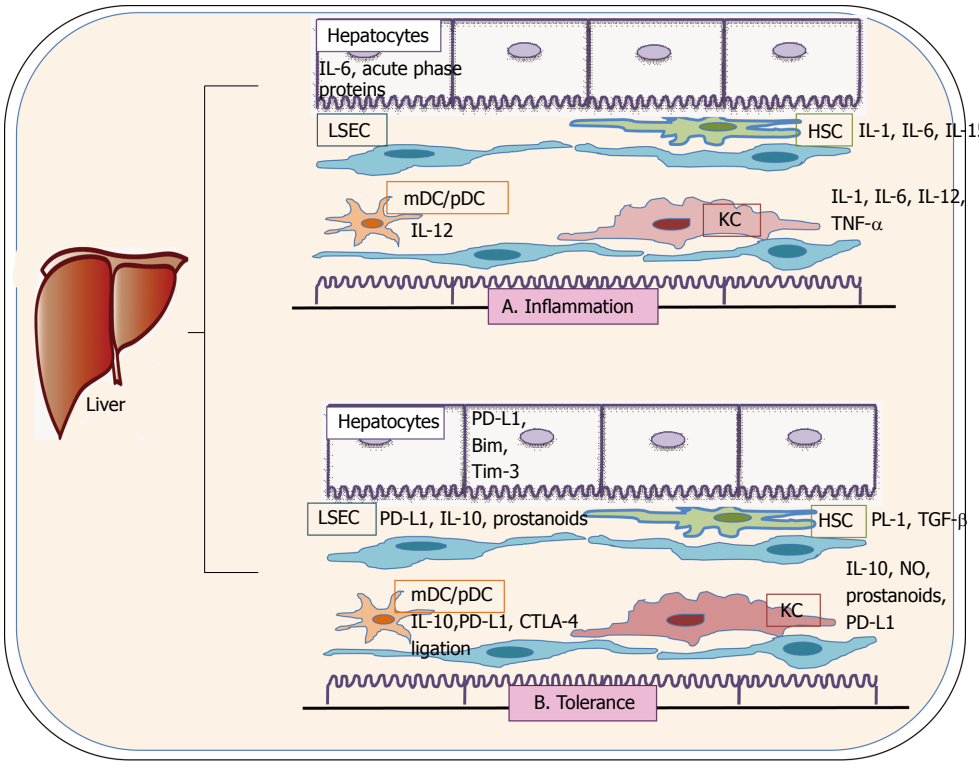

Figure 1 Collective illustration of the hepatic cells with inflammatory and tolerance activities by stimulation of different molecules or receptors. LSEC: Liver sinusoidal endothelial cells; KC: Kuffer cells; DC: Dendritic cells; HSC: Hepatic stellate cells; TNF: Tumor necrosis factor; IL: Interleukin; mDC: Myeloid dendratic cell; pDC: Plasmacytoid dendritic cell; PD-L1: Programmed death ligand-1; Bim: BCL-2 interacting mediator; Tim-3: T cell immunoglobulin mucin-3; CTLA-4: Cytotoxic T-lymphocyte antigen 4; TGF: Transforming growth factor; NO: Nitric oxide.

could be due to large number of activated T cells[24].

Naïve T cells activation in the liver

The site of T cell activation is a determinant of the outcome of an immune response in the liver[22]. Tolerance will occur when T cells are activated in the liver. On the other hand, an effective immune response will be generated, when T cells are activated in the lymph nodes. This model put forward the theory that tolerance during viral hepatitis could be the result of early deletion of antigen-specific T cells from the T cell repertoire in the liver[22]. Usually, naïve T cells are activated in secondary lymphoid organs with consequent up regulation of adhesion molecules and integrins expression, which can bind to endothelial layer of the target organ and ultimately direct T cells to the parenchyma[25]. Moreover, T cells are not able to interact with parenchymal cells easily and thus they are not usually activated in the solid organs. In spite of this, the situation in the liver is slightly different. Fenestrated endothelial layer in the liver makes available interactions between naïve T cells and liver cells[26]. It has been showed by MHC class I -restricted, hepatitis B surface Ag-specific CD8+ polyclonal CTL adoptively transferred into wide-spread antigen expressing transgenic mouse model, leading to retention of those cells within the liver[26]. Moreover, it has been shown that primary antigen-specific T cell can be activated in the liver independently of lymphoid tissues[27].

Liver APCs in tolerance

Retention, activation and tolerance of naïve T cells in the liver is the result of the action of resident liver cells, including liver sinusoidal endothelial cells (LSEC), Kuffer cells (KC), liver dendritic cells (DC), hepatic stellate cells (HSC) and hepatocytes. Their collective function in induction of inflammatory response and tolerance has been illustrated in the Figure 1.

Endocytosis specialist-LSEC can express MHC class Ⅰ and Ⅱ, accessory CD80, CD86 and CD40 molecules. These features enable those cells to behave as potent APCs with the ability to activate both naïve CD4 and CD8 T cells as well as to cross-present exogenous antigen towards CD8 T cells[28]. However, LSEC primed naïve CD4+ T cells produce cytokines typical from Th0 rather than Th1 cells[29]. In addition, LSECs constitutively expressed ICAM-1, which helped in trapping of specific CD8+ T cells in the liver, resulting this process in activated T cell apoptosis[21]. Furthermore, the cross presentation of antigen by LSEC mainly leads to CD8+ T cells tolerance rather than immunity, demonstrating that LSEC-induced tolerance is an active and dynamic process[30].

Bone marrow derived and largest group of liver resident macrophages-KC mediate host resistance to infection. Interleukin (IL)-1, IL-6, IL-12 and tumor necrosis factor-α (TNF-α) pro-inflammatory cytokines released by KC[31] are involved in the inflammatory activities, whereas the nitric oxide, prostaglandin and IL-10 released by KC[29,32] down-regulate the production of pro-inflammatory cytokines and thereby may contribute to induction of hepatic tolerance. Furthermore, DC-induced antigen-specific T cell activation can be inhibited by KCs[29], which could also favor tolerance development. In addition, as in LSECs, KCs expressed ICAM-1 mediated trapping of specific CD8+ T cells in the liver resulting in activated T cell death[21].

Liver DCs are primarily located within periportal areas

and around central veins, which exert tolerogenic properties due to "immature" phenotype. The production of PD-1 and cytotoxic T lymphocyte antigen-4 (CTLA-4) by resting DCs, which are crucial negative co-stimulatory molecules, helps in inducing peripheral CD8+ T cell tolerance by inhibiting proliferation and cytokine production of liver infiltrating effector T cells[33]. In addition, liver generated DCs are more tolerogenic than DC in lymphatic tissue[34].

The role of HSCs in hepatic fibrosis includes stimulation of CD4, CD8+ T cells and NKT cells[35,36]. However, function of HSCs involves not only the inflammatory response[36], but also a tolerogenic role[37,38], which is the result of induction of T cell death[38] by intrinsic mechanism of immune inhibition. The HSCs regulate immune modulation by inducible B7-H1 expression, an inhibitor molecule of B7 family, resulting in T cell apoptosis induction.

Hepatocytes are also capable of activating naïve CD8+ T cells[39,40] and their interactions with CD8+ T cells may occur through LSEC fenestrations[38]. However, hepatocytes fail to promote activated CD8+ T cells survival, leading to an impaired T cell activation[39]. In addition, hepatocyte-activated T cells *in vitro* acquired activity and secrete cytokines but both levels are not constant and T cells consequently appeared to die by passive mechanisms[41]. Furthermore, infiltrating CD4+ T cells differentiate into a less inflammatory phenotype due to the interaction with MHC Ⅱ-expressing hepatocytes, which also helps to abrogate antiviral CD8+ T-cell response and viral clearance[42], which conclude in the tolerance during infection. It has been already proved that T cells activated by hepatocytes undergo premature death[43], whereas naïve CD8+ T cells priming by DC in the lymph nodes acquired effector functions in the liver.

The site of primary T cell activation could also induce emperipolesis of CD8+ T cells in the liver[43], which leads to non-apoptotic, destruction of these CD8+ T cells after degradation by lysosomal proteolytic enzymes. This distinct form of emperipolesis has been termed as "suicidal emperipolesis" (SE)[44]. Benseler *et al*[44] suggested that SE is a significant mechanism by which death of activated naïve CD8+ T cells occur in the liver within the first few hours before T cells are able to divide and expand. It is also involved in maintenance of tolerance, which is reinforced by break of tolerance in immune-mediated liver damage by treatment of wortmannin[44], inhibitor of phosphoinositide 3-kinases that blocks emperipolesis. Therefore, SE is an extremely efficient mechanism, able to rapidly delete T cells.

T cell stimulation in the liver encourages tolerance by using mechanisms such as, immune divergence[45], generation of regulatory T cells[46], T cell anergy[47] and T cell death[1]. Undeniably, hepatic tolerance can explain the elevated frequency of viral persistence during hepatotropic virus infections[1]. Although there are evidences showing that most infectious microorganisms are promptly removed from the liver, a favorable situation for evading immune responses occurs in some viruses, leading to the triumph of certain pathogens such as HBV and HCV. Till date, there are two main mechanisms by which HBV and HCV could successfully escape from CTL action: escape mutant generation, and immunosuppressive effects exertion (effector T cell exhaustion and T cell death by apoptosis)[2,48-50]. Among these mechanisms involved in viral hepatitis persistence, new advances on the role of T cell death induction have been obtained recently and our review in the apoptosis role, paying special attention to the last new insights in this issue will be discussed in the following pages.

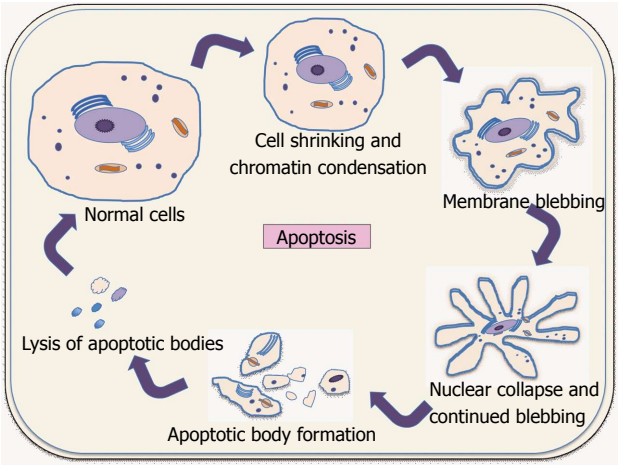

Figure 2 Apoptosis-programmed cell death.

APOPTOSIS

A normal cellular process involving physiologically relevant cell death and deletion of unwanted cells is called apoptosis. Apoptosis is essential for cell selection, tissue homeostasis, morphogenesis, and host defense in multicellular organisms. A cell that undergoes apoptosis dies neatly, without damaging its neighbors. The apoptotic signals give rise to activate various proteins and follow a specific classical caspase chain reaction set activation[51]. Quickly and neatly dismantlement process includes membrane blebbing with shrinking of the cytoplasm and condensation of the nucleus. Phagocytic cells begin to pick up the apoptotic bodies, preventing the release of cellular content and ultimately avoiding inflammation[52] (Figure 2). Apoptosis occurs by two mechanisms: active and passive mechanism. No presence of antigen gives a signal for termination of immune response by passive apoptotic mechanism (intrinsic pathway). On the other hand, the ligation of Fas (CD95) and TNF receptors-"death receptors" triggered apoptosis lead to active mechanism of apoptosis (extrinsic pathway). Briefly, apoptosis mechanisms involve a family of cysteine proteases, called caspases. These molecules are synthesized in the cell as inactive precursors, or pro-caspases for self-protection against accidental death, which are usually activated after receiving proper trigger by cleavage (Figure 3). Structurally, pro-caspases contain three domains: N terminal prodomain, a large subunit

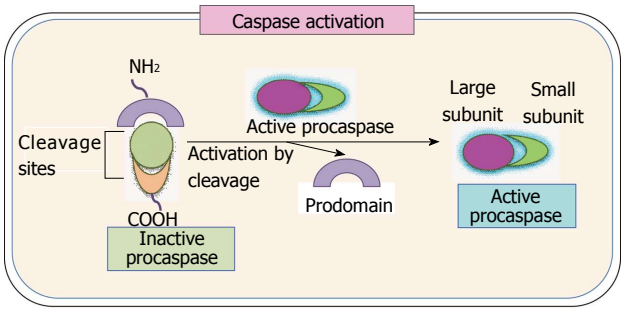

Figure 3 Caspase activation: Inactive proenzyme (procaspase) activated by proteolytic cleavage by another member of caspase family and cleaved two fragments associate to form the active site of the caspase.

and a small subunit. After activation, the active caspase enzyme is formed by heterodimerization of small and large subunits[43]. Moreover, active caspase molecules are ready to cleave target proteins such as structural or signaling proteins and other effector caspases, preventing other proteins cleavage randomly[52].

Extrinsic pathway

The extrinsic pathway initiates from outside the cell through triggering the activation of transmembrane "death receptors" that are members of the TNF receptor gene superfamily. Members of this receptor family bind to extrinsic ligands known as pro-apoptotic ligands[53] and transduce intracellular signals that ultimately result in the destruction of the cell[54,55]. To date the most well characterized ligands of these receptors are FasL, TNF-α, Apo3L and Apo2L and corresponding receptors are FasR, TNFR1, DR3 and DR4/DR5, respectively[55-57]. The signal transduction of active cell death process involves several caspases. Activated caspases have an effect on several cellular functions as part of the process that results in the death of the cells[53].

The signal transduction of mitochondrial-independent active cell death process involves binding of a pro-apoptotic ligand (such as FasL) with its receptors (Fas) on the surface of a target cell. The cytosolic tail of receptors contains a death domain, which when activated, binds to an adaptor protein, which in turn recruits the specific procaspase-8 and -10 and activates them by proteolytic cleavage[58] that finally initiates the proteolytic caspase cascade leading to apoptosis. Activated caspase 8 triggers the caspase cascade *via* two different pathways, leading to cell death. In type 1 apoptosis, such as in lymphocytes, caspase 8 activates caspase 3 whereas in type 2 apoptosis, like in hepatocytes and pancreatic cells, caspase 8 activate the pro-apoptotic molecule Bid and go ahead for apoptosis *via* the disruption of mitochondrial membrane and cytochrome C release[59] (Figure 4). The T cell death by type 1 and type 2 Fas induced apoptosis fate is decided by the ratio between proteolytically activated effector caspases, X-chromosome linked inhibitor of apoptosis protein and proto-typical effector caspase substrate inhibitor of caspase-activated DNase. Interestingly, HCV specific intrahepatic lymphocytes contribute to bystander killing *via* Fas-FasL interaction[60], which support the fact that the liver facilitates liver-trapped activated T cell apoptosis[61].

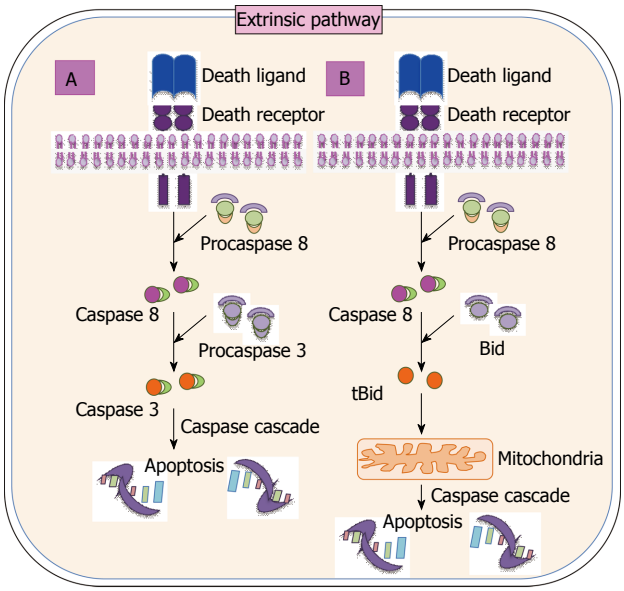

Figure 4 Extrinsic pathway. A: Mitochondria-independent extrinsic pathway: Fas-FasL ligation strikes to recruit pro-caspase 8 activation and induction of caspase cascade by caspase 3 leading to apoptosis; B: Mitochondria-dependent extrinsic pathway: Fas-FasL ligation trigger to activate the pro-caspase 8, which cleave Bid (pro-apoptotic Bcl-2 family molecule) to form truncated Bid (tBid). Then, mitochondrial dependent cell death begins with tBid.

Intrinsic pathway

The intrinsic or mitochondrial pathway is initiated within the cell, involving non-receptor-mediated intracellular signals and inducing activities in the mitochondria that initiate apoptosis. DNA damage, loss of cell-survival factors or other types of severe cell stress causes the induction signal for the intrinsic pathway. This passive death process pivots on the balance of activity between pro- and anti-apoptotic signals of the B cell lymphoma 2 (Bcl-2) family proteins[62]. This balance is maintained by regulation of the permeability of the mitochondrial membrane and by the pro- or anti-apoptotic signal that will be released inside the cell[63]. Following mitochondrial permeabilization, the intrinsic pathway divides into two pathways: Apoptosis protease-activating factor-1 (Apaf-1) dependent and Apaf-1 independent pathway. In Apaf-1 dependent pathway, release of cytochrome c from mitochondria, by triggering the pro-apoptotic Bcl-2 family member[64], and ATP activate monomer inactive Apaf-1 proteins by a conformational change, leading to form a heptamer of Apaf-1 molecules called apoptosome[65]. Apoptosome allows activation of pro-caspase 9, which consequently triggers the caspase cascade[66]. On the other hand, in Apaf-1 independent pathway, permeabilization of mitochondrial membrane release DIABLO like proteins, which activates effector caspases by provoking inhibitors of apoptosis proteins[67] and triggers caspase cascade[68] (Figure 5).

The balance of pro- and anti-apoptotic proteins main-

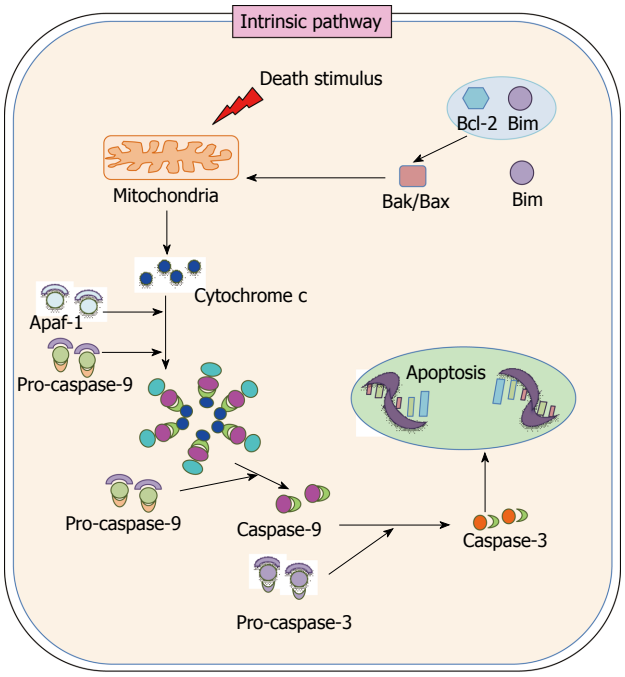

Figure 5 Intrinsic pathway. Death stimulation up regulates Bcl-2 interacting mediator leading to the separation from Bcl-2, favoring the activation of Bax, Bak, which form pores in the mitochondrial membrane leading to release of cytochrome c. Cytochrome c with Apaf-1 and procaspase 9 participate in the formation of apoptosome, which activate caspase 9. Caspase-9 activates caspase 3 after cleavage of pro-caspase-3. That caspase-3 triggers to induction of caspase cascade and cell death. Apaf-1: Apoptosis protease-activating factor-1. Bim: Bcl-2 interacting mediator.

tains the apoptotic activity[69]. The Bcl-2 family members regulate mostly neglect or intrinsic pathway. This family is subdivided into three groups of proteins on the basis of their functions and the number of Bcl-2 homology (BH) motifs included in their primary structure; first group: "anti-apoptotic multidomain" members, such as Bcl-xL, have four BH domains (BH1 to BH4) which inhibits apoptotic process. Other two groups of "pro-apoptotic multidomain" members, which are Bax-like proteins and "BH3-only" proteins[70]. Bax-like proteins possess threee BH domain (BH1 to BH3), including Bax, Bak, and Bok, which are referred as death effector members. BH3-only members contain BH3 domain, including Bim, Bad, Bik, Puma, Noxa and Bid and are known as messengers of death. In addition, C-terminal transmembrane (TM) fragment is thought to confer anchorage to mitochondrial membranes, which is also possessed by most multi-BH members and several BH3-only proteins.

Three models (Figure 6) have been postulated by which the BH3 family promotes passive cell death in which Bax and Bak bind directly or indirectly with cell death sensitizer (*e.g.*, Bad, Bik) and activators of cell death (*e.g.*, Bim, tBid). The direct activation model proposes that sensitizer BH3-only proteins displace the activator BH3-only proteins from the anti-apoptotic proteins to promote apoptosis. Anti-apoptotic proteins inhibit the activator BH3-only proteins but not Bax and Bak to suppress apoptosis. In the displacement model, Bax and Bak are sequestered by anti-apoptotic proteins for cell survival and constitutively active in cells. BH3-only proteins play the sensitizer role and inhibit their respective anti-apoptotic proteins to promote apoptosis. The third model, called embedded together model, highlights the interactions occurring in and on membranes, which were not explained by direct activation and displacement model. In embedded together model, Bcl-2 family proteins insert into and change their conformations according to their functions in membrane[71]. The predominantly studied messenger death molecule, Bcl-2 interacting protein (Bim) will be focused further.

BIM

Bim/Bod is a pro-apoptotic protein belonging to the BH3-only group of Bcl-2 family members and is being called the "ghost" molecule or "suicide" molecule, which enables cells to expire gracefully. Two independent studies discovered Bim as a Bcl-2 binding protein and Mcl1-binding protein in 1998[72,73]. Bim induces apoptosis by binding to and antagonizing anti-apoptotic members of the Bcl-2 family. The Bim interactions have been observed with Bcl-2 family members, such as Bcl-2, Bcl-xL, Mcl-1, Bcl-w, *etc*[72,73].

Bim is a well-known pivotal initiator of apoptosis in thymocyte-negative selection[74]. Bim has 19 Bim isoforms including three major isoforms, which have distinct sizes and pro-apoptotic activities in the mammals, caused by alternative splicing: BimEL (extra long), BimL (long) and BimS (small)[73]. The shortest form, BimS, is the most potent and is generally only transiently expressed during apoptosis[73]. The other two isoforms are sequestered to the dynein motor complex, and apoptotic activity of these longer isoforms is regulated by phosphorylation[75,76], which is triggered by environmental stress, resulting in its dissociation from the dynein complex and increasing apoptotic activity.

Expression of Bim is up regulated in human T cells in response to TCR-triggering by protein kinase C and calcineurin pathways[77]. Nevertheless, there are other mechanisms involved in Bim up-regulation during chronic infection, such as the effect of certain cytokines. In fact, in a persistent viral infection animal model, Bim-mediated apoptosis correlates with low IL-7 receptor expression on specific T cells[78].

The regulation of Bim expression at transcriptional level in growth factor deprivation and in endoplasmic reticulum stress has observed by the class O fork-head box transcription factor (FOX03A) and transcriptional factor CEPB-α respectively[79,80]. Post-transcriptional phosphorylation of Bim can also regulate its function. Phosphorylated Bim is targeted for proteasomal degradation and avoid its interaction with Bax, thus maintaining cell existence[81,82]. The signaling adaptor TNFR-associated factor 1 (TRAF1) negatively correlates with Bim and it contributes to CD8 T cell-mediated control of chronic viral infections. In addition, linking between survival

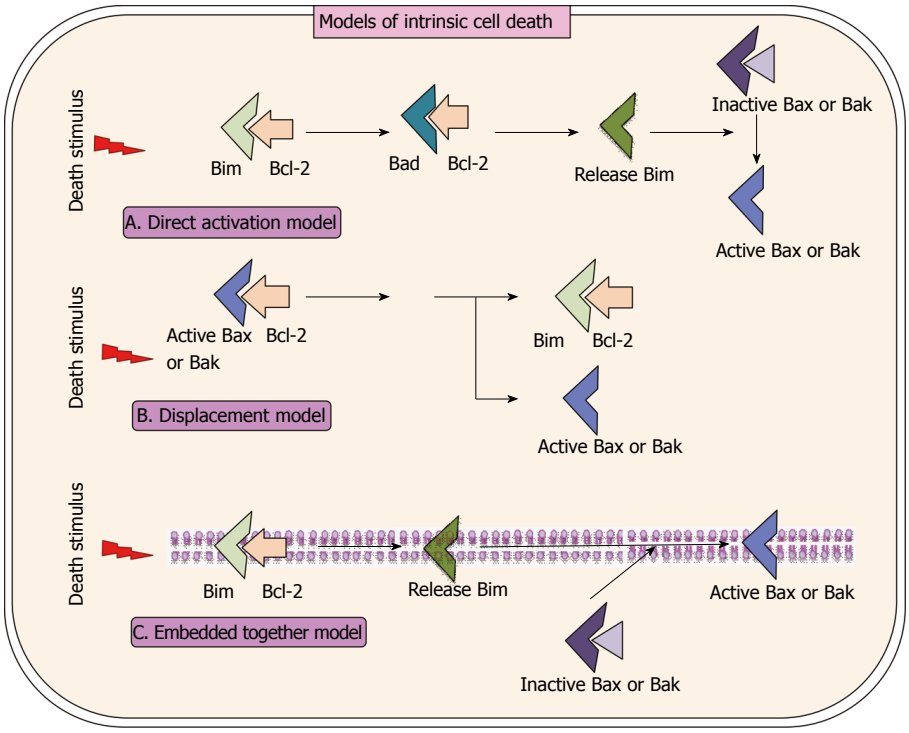

Figure 6 Models for intrinsic cell death. A: Direct activation model postulates Bcl-2 interacting mediator (Bim) is required for activating Bax and Bak. Anti-apoptotic proteins inhibit BH3-only proteins to suppress apoptosis, but not Bax or Bak. Replacement of Bim to sensitizer BH3-proteins from the anti-apoptotic proteins occurs to promote apoptosis; B: The displacement model proposes that anti-apoptotic proteins for cell survival must sequester constitutively active Bax and Bak in cells. Bim inhibits their respective anti-apoptotic proteins by playing sensitizer role to promote apoptosis; C: Embedded together model highlights the active role of the membrane, which is not defined in direct activation model and displacement model. Bcl-2 family proteins insert into and change their conformations that dictate their functions at the membrane. Sensitizer BH3-only proteins relocate the activator BH3-only proteins and Bax/Bak from the anti-apoptotic proteins to endorse apoptosis. Activator BH3-only proteins recruit Bax to the membrane to induce mitochondrial outer membrane permeabilization and apoptosis. These reversible interactions are directed by equilibrium constants that are depended on the concentrations and interactions of the proteins with each other and with membranes.

effects of TRAF1 and TRAF1-dependent Bim down-modulation has been shown in CD8 T cells[83-85]. TRAF1 is particularly vanished from virus-specific CD8 T cells during the chronic human immunodeficiency virus and lymphocytic chorio-meningitis virus (LCMV) infection[86].

Bim plays a vital role in the immune system, in bone biology and in tumor-genesis by inducing apoptosis[87]. Bim in T cells, B cells, neurons and many other cell types can trigger apoptosis[87]. Gene targeting in mice for the important region for apoptosis, BH3 region, uncovered the important physiological role in Bim[88]. In fact, in the absence of Bim leukocytes in blood as well as in LNs, thymus, spleen were high in number[88]. The role of Bim in apoptosis has been revealed in Bim-/- thymocytes, which were more resistant to apoptosis after different apoptotic treatment such as ionomycin, taxol, γ irradiation[88].

DEATH OF ACTIVATED T CELLS BY BIM

The liver is having a property that might explain its role in inducing tolerance due to its recognition as an alternative primary activation of CD8 T cells site. The phenotype of activated CD8 cells in the liver was the same as in lymph nodes. However, liver-activated CD8 T cells displayed poor effector functions and a unique $CD25^{low}$ $CD54^{low}$ phenotype, which was associated with increased expression of the Bim and caspase-3, demonstrating that these cells are programmed to apoptosis following intrahepatic activation. Strikingly, Bim deficient T cells survived following intrahepatic activation[1]. Therefore, the phenotype and fate of naïve CD8 T cells activated by hepatocytes *in vivo* could explain the death penalty role of Bim in chronic hepatotropic viral infection[1]. The distinct phenotype can be due to the lack of co-stimulatory molecule expression on hepatocytes[43]; however the treatment with IL-2 or anti-CD28 antibodies could rescue hepatocyte-activated cells from death[41].

Lymphocyte fate deciding pathways synergize to kill activated T cells in chronic herpes simplex viral immune responses, whereas death of activated T cells in acute immune responses relies only on the mitochondrial pathway involved only Bim with no contribution by Fas, which showed critical overlapping roles for Fas and Bim in T cell death during immune response shutdown, leading to immune tolerance[23].

BIM IN HEPATITIS

Bim has been shown to be important for CD8 T cell viability during chronic LCMV infection in mice[89]. In this study, in Bim mutated mice, Bim mutation almost completely blocked the deletion of cognate antigen specific CD8 T cells in liver during chronic viral infection. Bim has a critical role in maintaining naive and memory T cells in LCMV infection[90]. In another study, it has been shown that a defect in apoptosis dramatically not only enhances the antigen-specific memory T cells but also increased the number of virus-specific CD4+ T cells in the lymph nodes following acute LCMV infection, compared to the parental genotypes or wild type mice[91]. Therefore, the loss of both Bim and Fas caused the increase in memory T cells in acute LCMV infection[91]. The Bim role has been demonstrated in the development of LCMV-induced, T cell-mediated hepatitis by controlling the apoptosis of both T cells and hepatocytes[92].

Bim attrition of virus specific CTLs during HBV

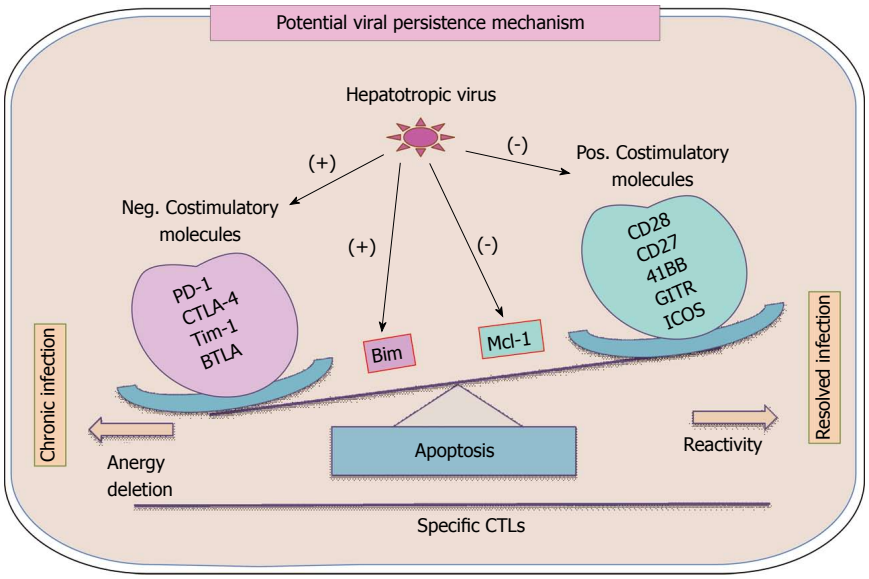

Figure 7 Balance between co-stimulatory/apoptotic molecules and viral-specific cytotoxic T lymphocytes reactivity according to infection outcome. Neg.: Negative; Pos.: Positive; CTLs: Cytotoxic T lymphocytes; (+): Possible molecules induced by viral infection; (-): Possible molecules down-regulated by viral infection; BIM: Bcl-2 interacting mediator; Mcl-1: Myeloid cell leukemia sequence-1.

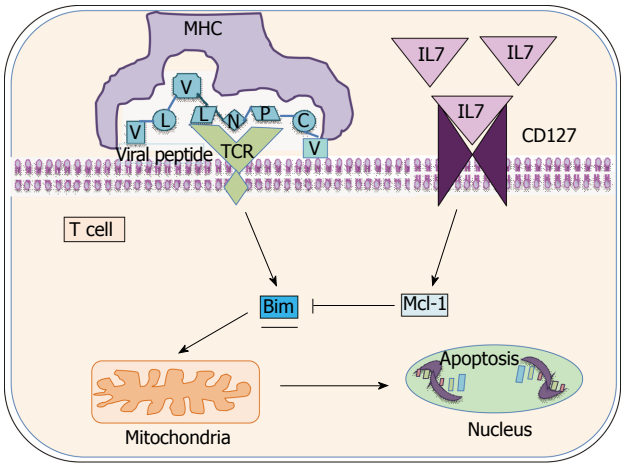

Figure 8 Cell survival marker CD127 modulates Bim and myeloid cell leukemia sequence-1 expression on hepatitis C virus-specific cytotoxic T lymphocytes after cognate antigen stimulation. Misbalance of Mcl-1/Bcl-2 interacting mediator (Bim) triggers to apoptosis of hepatitis C virus specific cytotoxic T lymphocytes. TCR: T cell receptor; Mcl-1: Myeloid cell leukemia sequence-1.

infection has also been confirmed[3,93]. The gene expression profile in HBV infection showed different patterns of gene expression on HBV-specific CD8+ T cells according to viral control. Bim was one of the up-regulated genes in HBV-specific CD8+ T cells from patients with chronic HBV infection. Blocking Bim-mediated apoptosis improved recovery of HBV-specific CD8+ T cell function[3]. Furthermore, the elevated apoptosis has been observed not only with Bim tolerogenic phenotype, but also with co-inhibitory signals through CTLA-4[93] or T cell-intrinsic transforming growth factor-β[94].

As discussed earlier, robust CD8 responses are essential to control HCV infection. However, in HCV chronic infection, HCV specific CD8 are depleted by Bim mediated attrition, and remaining cells are functionally exhausted. The cell survival factor CD127 counteracts the induction of apoptosis after antigen encounter through myeloid cell leukemia sequence-1 (Mcl-1) expression and Bim down-regulation[95] after the cognate antigen recognition by TCR. Similarly, our group has shown in previous work, HCV-specific CTLs displayed a high Bim expression in persistent infection respect to resolved infection patients[2], suggesting a similar apoptotic mechanism to the one described in chronic HBV infection.

The procedure of T cell death during chronic viral infection is determined by a carefully balanced and complex group of pro- and anti-apoptotic proteins of the Bcl-2 family, such as Bim and Mcl-1[96] (Figure 7). Interestingly, persistent hepatotropic viral infection is characterized by continuous TCR triggering and CD127 down-regulation on viral-specific CTLs[97], which could favor Bim up-regulation. In addition, it is well known that Bim is clearly involved in intrahepatic specific-CTL apoptosis in animal models[1]. Furthermore, Bim pro-apoptotic effect is blocked by the action of Bcl-2 family anti-apoptotic proteins such as Mcl-1 and Bcl-2[78,98], clearly pointing out that T cell death also depends on the anti-apoptotic protein expression. Bearing in mind all these facts, recently our group has suggested a model to explain specific CTL deletion during persistent hepatotropic viral infection (Figure 8). This model shows that CD127 phenotype modulates Bim and Mcl-1 expression on virus-specific CTLs, leading to Mcl-1/Bim imbalance during persistent infection, which impairs T cell reactivity and suggesting that restoration of T cell function could occur by correcting the levels of Mcl-1 and Bim expression.

In our work, Bim up-regulation has been observed on $CD127^{low}$-expressing HCV-specific CTLs but not on $CD127^{high}$ cells after antigen encounter, suggesting that TCR triggering can only lead to Bim up-regulation in absence of IL-7 stimulation on HCV-specific CTLs. Nevertheless, Bim level is not enough to lead to T cell apoptosis. Our data also showed the Mcl-1/Bim ratio could decide the fate of the activated T cells by sequestration of experienced $CD127^{low}$/$Mcl\text{-}1^{low}$-expressing T cells to the liver and subsequent Bim up-regulation after antigen encounter due to the absence of IL-7 stimulus[99]. Finally, Bim

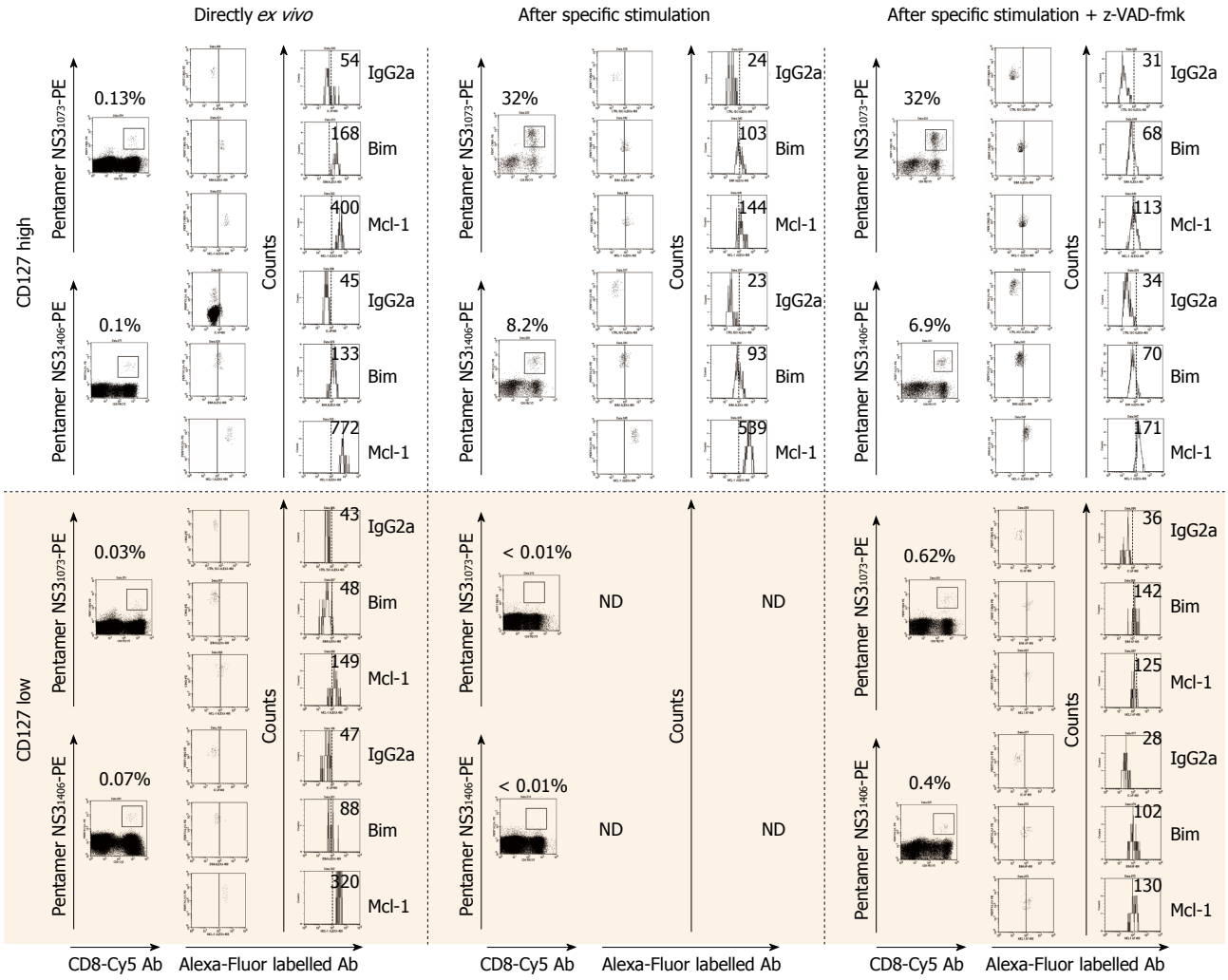

Figure 9 Figure illustrated the FACSan® dot plots and histograms from peripheral blood lymphocytes from two hepatitis C virus patients with different CD127 expression on hepatitis C virus-specific cytotoxic T lymphocytes (CD8+/Pentamer+ cells). The different plots show the Bcl-2 interacting mediator (Bim) and myeloid cell leukemia sequence-1 (Mcl-1) expression directly *ex vivo* and after specific stimulation on peripheral CD8+/pentameter+ cells according to CD127 level. The figure on the top of the dot-plots represents the frequency of penatmer+ cells out of total CD8+ cells. The figure in the upper right corner in the histogram plot represents the MFI for Bim and Mcl-1 staining. The continuous and dashed line in the dot-plots and histograms represents the cut-off point to consider a staining positive according to the negative control. ND: Not done due to lack of pentamer+/CD8+ proliferation after specific stimulation.

would be released freely to activate Bax, due to the low level of the anti-apototic protein Mcl-1 during chronic HCV infection. Consequently, CD127 level play a central role in hepatotropic virus-specific CD8+ T cell apoptosis by regulation of Mcl-1 expression *in vivo* and by Bim modulation after antigen encounter, which is checked by T cell reactivity restoration and Mcl-1/Bim phenotype on CD127^{low} specific CTLs after apoptosis blockade (Figure 9), that suggested a link between apoptosis after TCR triggering and low CD127 expression on experienced specific CTLs during persistent infection that could be related to Mcl-1/Bim imbalance.

Therefore, CD127 phenotype modulates Bim and Mcl-1 expression on specific CTLs and this affect to T cell reactivity through apoptosis regulation. Specifically, during chronic infection, Mcl-1/Bim imbalance could be involved on CD127^{low} specific CTL hyporeactivity, but it could be overcome by blocking apoptosis.

For control of hepatotropic viral infection is essential to develop a robust viral-specific cellular response. However, during chronic infection this response is altered, showing a pro-apoptotic phenotype due to the deprivation of IL-7 secondary to the low expression of CD127. Recently, it has been investigated that TRAF1 is a signal adapter for positive co-stimulatory receptors whose level depends on the action of IL-7 and inhibits the expression of the pro-apoptotic molecule Bim[86]. Therefore, in situations of deprivation of IL-7, action of TRAF1 could be impaired, favoring an imbalance between anti- and pro-apoptotic molecules. On the other hand, in an experimental model, IL-7 deprivation during stressing conditions leads to Mcl-1 down-regulation on T cells, conducting to T cell death that could be avoided by IL-7 treatment[100]. Consequently, strategies directed to block the pro-apoptotic effect of IL-7 deprivation should be designed to increase the effectiveness of CTL response restoration, by enhancing the TRAF1 and Mcl-1 expression level that could restore Bim/Mcl-1 balance. On of those strategies

could be short-term use of cyclosporine-A or FK506 could block the induction of the pro-apoptotic molecule Bim on CD127$^-$ cells[77]. This strategy could favor specific-CTL restoration during anti-viral treatments in combination with the standard of care. Another possible strategy to restore hepatotropic virus-specific CTL reactivity during chronic infection could be the administration of IL-7, in order to increase the stimulation of the reduced number of IL-7R molecules on specific CTLs, to modulate the balance between Bim and Mcl-1. In fact, in an animal model of cytotoxic T cell exhaustion, IL-7 treatment resulted in amplified cytokine production, increased T cell effector function, and viral clearance[101].

CONCLUSION

The deletion of hepatitis virus-specific CD8+ T cells is likely to represent the deregulation of the Bim pro-apoptotic pathway. The balance between pro- and anti-apoptotic molecules is critical for cell survival. The unavailability of appropriate survival marker modulates Bim and Mcl-1 expression on virus hepatitis-specific CTLs and this affect to T cell reactivity through apoptosis regulation. The level of those molecules is regulated by CD127 (IL-7R) expression, which is down-modulated during persistent infection. Consequently, Mcl-1/Bim imbalance could be the reason for the deletion of virus hepatitis-specific T cells, but it could be overcome by interruption of apoptosis. The interruption of this tolerizing mechanism may provide a new strategy to restore the balance between apoptotic molecules in order to achieve viral specific T cell immunity, as a future treatment strategy of chronic viral hepatitis.

REFERENCES

1 **Holz LE**, Benseler V, Bowen DG, Bouillet P, Strasser A, O' Reilly L, d'Avigdor WM, Bishop AG, McCaughan GW, Bertolino P. Intrahepatic murine CD8 T-cell activation associates with a distinct phenotype leading to Bim-dependent death. *Gastroenterology* 2008; **135**: 989-997 [PMID: 18619445 DOI: 10.1053/j.gastro.2008.05.078]

2 **Larrubia JR**, Benito-Martínez S, Miquel J, Calvino M, Sanz-de-Villalobos E, González-Praetorius A, Albertos S, García-Garzón S, Lokhande M, Parra-Cid T. Bim-mediated apoptosis and PD-1/PD-L1 pathway impair reactivity of PD1(+)/CD127(-) HCV-specific CD8(+) cells targeting the virus in chronic hepatitis C virus infection. *Cell Immunol* 2011; **269**: 104-114 [PMID: 21481848 DOI: 10.1016/j.cellimm.2011.03.011]

3 **Lopes AR**, Kellam P, Das A, Dunn C, Kwan A, Turner J, Peppa D, Gilson RJ, Gehring A, Bertoletti A, Maini MK. Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection. *J Clin Invest* 2008; **118**: 1835-1845 [PMID: 18398508 DOI: 10.1172/JCI33402]

4 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]

5 **Tsukuma H**, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797-1801 [PMID: 7684822 DOI: 10.1056/NEJM199306243282501]

6 **Wherry EJ**, Barber DL, Kaech SM, Blattman JN, Ahmed R. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc Natl Acad Sci USA* 2004; **101**: 16004-16009 [PMID: 15505208 DOI: 10.1073/pnas.0407192101]

7 **Zajac AJ**, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* 1998; **188**: 2205-2213 [PMID: 9858507 DOI: 10.1084/jem.188.12.2205]

8 **Gruener NH**, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, Walker B, Sullivan J, Phillips R, Pape GR, Klenerman P. Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus. *J Virol* 2001; **75**: 5550-5558 [PMID: 11356962 DOI: 10.1128/JVI.75.12.5550-5558.2001]

9 **Xibing G**, Xiaojuan Y, Zhonghua L, Juanhua W. Alteration in cellular immunity after chronic hepatitis B deteriorated into severe hepatitis and its significance. *Hepat Mon* 2011; **11**: 810-815 [PMID: 22224079]

10 **Zinkernagel RM**, Haenseler E, Leist T, Cerny A, Hengartner H, Althage A. T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class I-restricted virus-specific cytotoxic T cells as a physiological correlate of the 51Cr-release assay? *J Exp Med* 1986; **164**: 1075-1092 [PMID: 3489805 DOI: 10.1084/jem.164.4.1075]

11 **Sewell AK**, Price DA, Oxenius A, Kelleher AD, Phillips RE. Cytotoxic T lymphocyte responses to human immunodeficiency virus: control and escape. *Stem Cells* 2000; **18**: 230-244 [PMID: 10924089 DOI: 10.1634/stemcells.18-4-230]

12 **Dustin LB**, Rice CM. Flying under the radar: the immunobiology of hepatitis C. *Annu Rev Immunol* 2007; **25**: 71-99 [PMID: 17067278 DOI: 10.1146/annurev.immunol.25.022106.141602]

13 **Zhang P**, Zhong L, Struble EB, Watanabe H, Kachko A, Mihalik K, Virata-Theimer ML, Alter HJ, Feinstone S, Major M. Depletion of interfering antibodies in chronic hepatitis C patients and vaccinated chimpanzees reveals broad cross-genotype neutralizing activity. *Proc Natl Acad Sci USA* 2009; **106**: 7537-7541 [PMID: 19380744 DOI: 10.1073/pnas.0902749106]

14 **Maini MK**, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D, Naoumov NV, Ferrari C, Bertoletti A. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191**: 1269-1280 [PMID: 10770795 DOI: 10.1084/jem.191.8.1269]

15 **Fuller MJ**, Khanolkar A, Tebo AE, Zajac AJ. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. *J Immunol* 2004; **172**: 4204-4214 [PMID: 15034033]

16 **Maini MK**, Boni C, Ogg GS, King AS, Reignat S, Lee CK, Larrubia JR, Webster GJ, McMichael AJ, Ferrari C, Williams R, Vergani D, Bertoletti A. Direct ex vivo analysis of hepatitis B virus-specific CD8(+) T cells associated with the control of infection. *Gastroenterology* 1999; **117**: 1386-1396 [PMID: 10579980 DOI: 10.1016/S0016-5085(99)70289-1]

17 **Calne RY**, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, Davies DA. Induction of immunological tolerance by porcine liver allografts. *Nature* 1969; **223**: 472-476 [PMID: 4894426 DOI: 10.1038/223472a0]

18 **Crispe IN**, Giannandrea M, Klein I, John B, Sampson B, Wuensch S. Cellular and molecular mechanisms of liver tolerance. *Immunol Rev* 2006; **213**: 101-118 [PMID: 16972899 DOI: 10.1111/j.1600-065X.2006.00435.x]

19 **Huang L**, Soldevila G, Leeker M, Flavell R, Crispe IN. The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. *Immunity* 1994; **1**: 741-749 [PMID: 7895163 DOI: 10.1016/S1074-7613(94)80016-2]

20 **Chase MW**. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. *Proc Soc Exp Biol Med* 1946; **61**: 257-259 [PMID: 21024163]

21 **Mehal WZ**, Juedes AE, Crispe IN. Selective retention of acti-

vated CD8+ T cells by the normal liver. *J Immunol* 1999; **163**: 3202-3210 [PMID: 10477588]
22 **Bowen DG**, McCaughan GW, Bertolino P. Intrahepatic immunity: a tale of two sites? *Trends Immunol* 2005; **26**: 512-517 [PMID: 16109501 DOI: 10.1016/j.it.2005.08.005]
23 **Hughes PD**, Belz GT, Fortner KA, Budd RC, Strasser A, Bouillet P. Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. *Immunity* 2008; **28**: 197-205 [PMID: 18275830 DOI: 10.1016/j.immuni.2007.12.017]
24 **Qian S**, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE. Murine liver allograft transplantation: tolerance and donor cell chimerism. *Hepatology* 1994; **19**: 916-924 [PMID: 8138266 DOI: 10.1002/hep.1840190418]
25 **Mackay CR**, Marston WL, Dudler L. Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990; **171**: 801-817 [PMID: 2307933 DOI: 10.1084/jem.171.3.801]
26 **Ando K**, Guidotti LG, Cerny A, Ishikawa T, Chisari FV. CTL access to tissue antigen is restricted in vivo. *J Immunol* 1994; **153**: 482-488 [PMID: 8021489]
27 **Bertolino P**, Bowen DG, McCaughan GW, Fazekas de St Groth B. Antigen-specific primary activation of CD8+ T cells within the liver. *J Immunol* 2001; **166**: 5430-5438 [PMID: 11313380]
28 **Limmer A**, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. *Nat Med* 2000; **6**: 1348-1354 [PMID: 11100119 DOI: 10.1038/82161]
29 **Knolle PA**, Schmitt E, Jin S, Germann T, Duchmann R, Hegenbarth S, Gerken G, Lohse AW. Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. *Gastroenterology* 1999; **116**: 1428-1440 [PMID:10348827 DOI: 10.1016/S0016-5085(99)70508-1]
30 **Diehl L**, Schurich A, Grochtmann R, Hegenbarth S, Chen L, Knolle PA. Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. *Hepatology* 2008; **47**: 296-305 [PMID: 17975811 DOI: 10.1002/hep.21965]
31 **Racanelli V**, Rehermann B. The liver as an immunological organ. *Hepatology* 2006; **43**: S54-S62 [PMID: 16447271]
32 **You Q**, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. *Hepatology* 2008; **48**: 978-990 [PMID: 18712788 DOI: 10.1002/hep.22395]
33 **Probst HC**, McCoy K, Okazaki T, Honjo T, van den Broek M. Resting dendritic cells induce peripheral CD8+ T cell tolerance through PD-1 and CTLA-4. *Nat Immunol* 2005; **6**: 280-286 [PMID: 15685176 DOI: 10.1038/ni1165]
34 **Pillarisetty VG**, Shah AB, Miller G, Bleier JI, DeMatteo RP. Liver dendritic cells are less immunogenic than spleen dendritic cells because of differences in subtype composition. *J Immunol* 2004; **172**: 1009-1017 [PMID: 14707074]
35 **Viñas O**, Bataller R, Sancho-Bru P, Ginès P, Berenguer C, Enrich C, Nicolás JM, Ercilla G, Gallart T, Vives J, Arroyo V, Rodés J. Human hepatic stellate cells show features of antigen-presenting cells and stimulate lymphocyte proliferation. *Hepatology* 2003; **38**: 919-929 [PMID: 14512879]
36 **Winau F**, Hegasy G, Weiskirchen R, Weber S, Cassan C, Sieling PA, Modlin RL, Liblau RS, Gressner AM, Kaufmann SH. Ito cells are liver-resident antigen-presenting cells for activating T cell responses. *Immunity* 2007; **26**: 117-129 [PMID: 17239632 DOI: 10.1016/j.immuni.2006.11.011]
37 **Benten D**, Kumaran V, Joseph B, Schattenberg J, Popov Y, Schuppan D, Gupta S. Hepatocyte transplantation activates hepatic stellate cells with beneficial modulation of cell engraftment in the rat. *Hepatology* 2005; **42**: 1072-1081 [PMID: 16250034 DOI: 10.1002/hep.20889]
38 **Chen CH**, Kuo LM, Chang Y, Wu W, Goldbach C, Ross MA, Stolz DB, Chen L, Fung JJ, Lu L, Qian S. In vivo immune modulatory activity of hepatic stellate cells in mice. *Hepatology* 2006; **44**: 1171-1181 [PMID: 17058227 DOI: 10.1002/hep.21379]
39 **Nishimura T**, Ohta A. A critical role for antigen-specific Th1 cells in acute liver injury in mice. *J Immunol* 1999; **162**: 6503-6509 [PMID: 10352265]
40 **Sakaguchi S**, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008; **133**: 775-787 [PMID: 18510923 DOI: 10.1016/j.cell.2008.05.009]
41 **Bertolino P**, Trescol-Biémont MC, Thomas J, Fazekas de St Groth B, Pihlgren M, Marvel J, Rabourdin-Combe C. Death by neglect as a deletional mechanism of peripheral tolerance. *Int Immunol* 1999; **11**: 1225-1238 [PMID: 10421780 DOI: 10.1093/intimm/11.8.1225]
42 **Wiegard C**, Wolint P, Frenzel C, Cheruti U, Schmitt E, Oxenius A, Lohse AW, Herkel J. Defective T helper response of hepatocyte-stimulated CD4 T cells impairs antiviral CD8 response and viral clearance. *Gastroenterology* 2007; **133**: 2010-2018 [PMID: 17967458 DOI: 10.1053/j.gastro.2007.09.007]
43 **Bowen DG**, Zen M, Holz L, Davis T, McCaughan GW, Bertolino P. The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. *J Clin Invest* 2004; **114**: 701-712 [PMID: 15343389]
44 **Benseler V**, Warren A, Vo M, Holz LE, Tay SS, Le Couteur DG, Breen E, Allison AC, van Rooijen N, McGuffog C, Schlitt HJ, Bowen DG, McCaughan GW, Bertolino P. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. *Proc Natl Acad Sci USA* 2011; **108**: 16735-16740 [PMID: 21933957 DOI: 10.1073/pnas.1112251108]
45 **Klugewitz K**, Blumenthal-Barby F, Schrage A, Knolle PA, Hamann A, Crispe IN. Immunomodulatory effects of the liver: deletion of activated CD4+ effector cells and suppression of IFN-gamma-producing cells after intravenous protein immunization. *J Immunol* 2002; **169**: 2407-2413 [PMID: 12193708]
46 **Lüth S**, Huber S, Schramm C, Buch T, Zander S, Stadelmann C, Brück W, Wraith DC, Herkel J, Lohse AW. Ectopic expression of neural autoantigen in mouse liver suppresses experimental autoimmune neuroinflammation by inducing antigen-specific Tregs. *J Clin Invest* 2008; **118**: 3403-3410 [PMID: 18802476]
47 **Goubier A**, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, Trinchieri G, Kaiserlian D. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* 2008; **29**: 464-475 [PMID: 18789731 DOI: 10.1016/j.immuni.2008.06.017]
48 **Bassett SE**, Di Bisceglie AM, Bacon BR, Sharp RM, Govindarajan S, Hubbard GB, Brasky KM, Lanford RE. Effects of iron loading on pathogenicity in hepatitis C virus-infected chimpanzees. *Hepatology* 1999; **29**: 1884-1892 [PMID: 10347134 DOI: 10.1002/hep.510290623]
49 **Thimme R**, Lohmann V, Weber F. A target on the move: innate and adaptive immune escape strategies of hepatitis C virus. *Antiviral Res* 2006; **69**: 129-141 [PMID: 16413618 DOI: 10.1016/j.antiviral.2005.12.001]
50 **Thimme R**, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; **194**: 1395-1406 [PMID: 11714747 DOI: 10.1084/jem.194.10.1395]
51 **Akiyama T**, Dass CR, Choong PF. Bim-targeted cancer therapy: a link between drug action and underlying molecular changes. *Mol Cancer Ther* 2009; **8**: 3173-3180 [PMID: 19934277 DOI: 10.1158/1535-7163.MCT-09-0685]
52 **Rathmell JC**, Thompson CB. The central effectors of cell death in the immune system. *Annu Rev Immunol* 1999; **17**: 781-828 [PMID: 10358774 DOI: 10.1146/annurev.immunol.17.1.781]
53 **Ashkenazi A**. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nat Rev Cancer* 2002; **2**: 420-430 [PMID: 12189384 DOI: 10.1038/nrc821]

54 **Bazzoni F**, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996; **334**: 1717-1725 [PMID: 8637518 DOI: 10.1056/NEJM199606273342607]

55 **Elmore S**. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 2007; **35**: 495-516 [PMID: 17562483 DOI: 10.1080/01926230701320337]

56 **Ashkenazi A**, Holland P, Eckhardt SG. Ligand-based targeting of apoptosis in cancer: the potential of recombinant human apoptosis ligand 2/Tumor necrosis factor-related apoptosis-inducing ligand (rhApo2L/TRAIL). *J Clin Oncol* 2008; **26**: 3621-3630 [PMID: 18640940 DOI: 10.1200/JCO.2007.15.7198]

57 **Roy S**, Nicholson DW. Cross-talk in cell death signaling. *J Exp Med* 2000; **192**: F21-F25 [PMID: 11034612 DOI: 10.1084/jem.192.8.F21]

58 **Fernandes-Alnemri T**, Armstrong RC, Krebs J, Srinivasula SM, Wang L, Bullrich F, Fritz LC, Trapani JA, Tomaselli KJ, Litwack G, Alnemri ES. In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proc Natl Acad Sci USA* 1996; **93**: 7464-7469 [PMID: 8755496 DOI: 10.1073/pnas.93.15.7464]

59 **Jost PJ**, Grabow S, Gray D, McKenzie MD, Nachbur U, Huang DC, Bouillet P, Thomas HE, Borner C, Silke J, Strasser A, Kaufmann T. XIAP discriminates between type I and type II FAS-induced apoptosis. *Nature* 2009; **460**: 1035-1039 [PMID: 19626005 DOI: 10.1038/nature08229]

60 **Gremion C**, Grabscheid B, Wölk B, Moradpour D, Reichen J, Pichler W, Cerny A. Cytotoxic T lymphocytes derived from patients with chronic hepatitis C virus infection kill bystander cells via Fas-FasL interaction. *J Virol* 2004; **78**: 2152-2157 [PMID: 14747581 DOI: 10.1128/JVI.78.4.2152-2157.2004]

61 **Crispe IN**, Dao T, Klugewitz K, Mehal WZ, Metz DP. The liver as a site of T-cell apoptosis: graveyard, or killing field? *Immunol Rev* 2000; **174**: 47-62 [PMID: 10807506 DOI: 10.1034/j.1600-0528.2002.017412.x]

62 **Fernàndez V**, Hartmann E, Ott G, Campo E, Rosenwald A. Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. *J Clin Oncol* 2005; **23**: 6364-6369 [PMID: 16155021 DOI: 10.1200/JCO.2005.05.019]

63 **Mayer B**, Oberbauer R. Mitochondrial regulation of apoptosis. *News Physiol Sci* 2003; **18**: 89-94 [PMID: 12750442]

64 **Kluck RM**, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 1997; **275**: 1132-1136 [PMID: 9027315 DOI: 10.1126/science.275.5303.1132]

65 **Acehan D**, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol Cell* 2002; **9**: 423-432 [PMID: 11864614 DOI: 10.1016/S1097-2765(02)00442-2]

66 **Li P**, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997; **91**: 479-489 [PMID: 9390557 DOI: 10.1016/S0092-8674(00)80434-1]

67 **Hague A**, Paraskeva C. Apoptosis and disease: a matter of cell fate. *Cell Death Differ* 2004; **11**: 1366-1372 [PMID: 15354202 DOI: 10.1038/sj.cdd.4401497]

68 **Okada H**, Suh WK, Jin J, Woo M, Du C, Elia A, Duncan GS, Wakeham A, Itie A, Lowe SW, Wang X, Mak TW. Generation and characterization of Smac/DIABLO-deficient mice. *Mol Cell Biol* 2002; **22**: 3509-3517 [PMID: 11971981 DOI: 10.1128/MCB.22.10.3509-3517.2002]

69 **Oltvai ZN**, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 1993; **74**: 609-619 [PMID: 8358790 DOI: 10.1016/0092-8674(93)90509-O]

70 **Youle RJ**, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 2008; **9**: 47-59 [PMID: 18097445 DOI: 10.1038/nrm2308]

71 **Shamas-Din A**, Brahmbhatt H, Leber B, Andrews DW. BH3-only proteins: Orchestrators of apoptosis. *Biochim Biophys Acta* 2011; **1813**: 508-520 [PMID: 21146563 DOI: 10.1016/j.bbamcr.2010.11.024]

72 **Hsu SY**, Lin P, Hsueh AJ. BOD (Bcl-2-related ovarian death gene) is an ovarian BH3 domain-containing proapoptotic Bcl-2 protein capable of dimerization with diverse antiapoptotic Bcl-2 members. *Mol Endocrinol* 1998; **12**: 1432-1440 [PMID: 9731710 DOI: 10.1210/me.12.9.1432]

73 **O'Connor L**, Strasser A, O'Reilly LA, Hausmann G, Adams JM, Cory S, Huang DC. Bim: a novel member of the Bcl-2 family that promotes apoptosis. *EMBO J* 1998; **17**: 384-395 [PMID: 9430630 DOI: 10.1093/emboj/17.2.384]

74 **Bouillet P**, Purton JF, Godfrey DI, Zhang LC, Coultas L, Puthalakath H, Pellegrini M, Cory S, Adams JM, Strasser A. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* 2002; **415**: 922-926 [PMID: 11859372 DOI: 10.1038/415922a]

75 **Lei K**, Davis RJ. JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci USA* 2003; **100**: 2432-2437 [PMID: 12591950 DOI: 10.1073/pnas.0438011100]

76 **Putcha GV**, Le S, Frank S, Besirli CG, Clark K, Chu B, Alix S, Youle RJ, LaMarche A, Maroney AC, Johnson EM. JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. *Neuron* 2003; **38**: 899-914 [PMID: 12818176 DOI: 10.1016/S0896-6273(03)00355-6]

77 **Sandalova E**, Wei CH, Masucci MG, Levitsky V. Regulation of expression of Bcl-2 protein family member Bim by T cell receptor triggering. *Proc Natl Acad Sci USA* 2004; **101**: 3011-3016 [PMID: 14970329 DOI: 10.1073/pnas.0400005101]

78 **Wojciechowski S**, Jordan MB, Zhu Y, White J, Zajac AJ, Hildeman DA. Bim mediates apoptosis of CD127(lo) effector T cells and limits T cell memory. *Eur J Immunol* 2006; **36**: 1694-1706 [PMID: 16761315 DOI: 10.1002/eji.200635897]

79 **Dijkers PF**, Medema RH, Lammers JW, Koenderman L, Coffer PJ. Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. *Curr Biol* 2000; **10**: 1201-1204 [PMID: 11050388 DOI: 10.1016/S0960-9822(00)00728-4]

80 **Puthalakath H**, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N, Gotoh T, Akira S, Bouillet P, Strasser A. ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* 2007; **129**: 1337-1349 [PMID: 17604722 DOI: 10.1016/j.cell.2007.04.027]

81 **Harada H**, Quearry B, Ruiz-Vela A, Korsmeyer SJ. Survival factor-induced extracellular signal-regulated kinase phosphorylates BIM, inhibiting its association with BAX and proapoptotic activity. *Proc Natl Acad Sci USA* 2004; **101**: 15313-15317 [PMID: 15486085 DOI: 10.1073/pnas.0406837101]

82 **Luciano F**, Jacquel A, Colosetti P, Herrant M, Cagnol S, Pages G, Auberger P. Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the proteasome pathway and regulates its proapoptotic function. *Oncogene* 2003; **22**: 6785-6793 [PMID: 14555991 DOI: 10.1038/sj.onc.1206792]

83 **Sabbagh L**, Pulle G, Liu Y, Tsitsikov EN, Watts TH. ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival in vivo. *J Immunol* 2008; **180**: 8093-8101 [PMID: 18523273]

84 **Sabbagh L**, Srokowski CC, Pulle G, Snell LM, Sedgmen BJ, Liu Y, Tsitsikov EN, Watts TH. A critical role for TNF receptor-associated factor 1 and Bim down-regulation in CD8 memory T cell survival. *Proc Natl Acad Sci USA* 2006; **103**: 18703-18708 [PMID: 17116875 DOI: 10.1073/pnas.0602919103]

85 **Zhang B**, Wang Z, Li T, Tsitsikov EN, Ding HF. NF-kappaB2 mutation targets TRAF1 to induce lymphomagenesis. *Blood* 2007; **110**: 743-751 [PMID: 17405906 DOI: 10.1182/blood-2006-11-058446]

86 **Wang C**, McPherson AJ, Jones RB, Kawamura KS, Lin GH, Lang PA, Ambagala T, Pellegrini M, Calzascia T, Aidarus N, Elford AR, Yue FY, Kremmer E, Kovacs CM, Benko E, Tremblay C, Routy JP, Bernard NF, Ostrowski MA, Ohashi PS, Watts TH. Loss of the signaling adaptor TRAF1 causes CD8+ T cell dysregulation during human and murine chronic infection. *J Exp Med* 2012; **209**: 77-91 [PMID: 22184633 DOI: 10.1084/jem.20110675]

87 **Akiyama T**, Tanaka S. Bim: guardian of tissue homeostasis and critical regulator of the immune system, tumorigenesis and bone biology. *Arch Immunol Ther Exp* (Warsz) 2011; **59**: 277-287 [PMID: 21633919 DOI: 10.1007/s00005-011-0126-1]

88 **Bouillet P**, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Köntgen F, Adams JM, Strasser A. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 1999; **286**: 1735-1738 [PMID: 10576740 DOI: 10.1126/science.286.5445.1735]

89 **Grayson JM**, Weant AE, Holbrook BC, Hildeman D. Role of Bim in regulating CD8+ T-cell responses during chronic viral infection. *J Virol* 2006; **80**: 8627-8638 [PMID: 16912311 DOI: 10.1128/JVI.00855-06]

90 **Wojciechowski S**, Tripathi P, Bourdeau T, Acero L, Grimes HL, Katz JD, Finkelman FD, Hildeman DA. Bim/Bcl-2 balance is critical for maintaining naive and memory T cell homeostasis. *J Exp Med* 2007; **204**: 1665-1675 [PMID: 17591857]

91 **Weant AE**, Michalek RD, Crump KE, Liu C, Konopitski AP, Grayson JM. Defects in apoptosis increase memory CD8+ T cells following infection of Bim-/-Faslpr/lpr mice. *Cell Immunol* 2011; **271**: 256-266 [PMID: 21839428 DOI: 10.1016/j.cellimm.2011.07.003]

92 **Lauer C**, Brunner T, Corazza N. The proapoptotic Bcl-2 family member Bim plays a central role during the development of virus-induced hepatitis. *J Immunol* 2012; **188**: 916-922 [PMID: 22156338 DOI: 10.4049/jimmunol.1101864]

93 **Schurich A**, Khanna P, Lopes AR, Han KJ, Peppa D, Micco L, Nebbia G, Kennedy PT, Geretti AM, Dusheiko G, Maini MK. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. *Hepatology* 2011; **53**: 1494-1503 [PMID: 21360567 DOI: 10.1002/hep.24249]

94 **Tinoco R**, Alcalde V, Yang Y, Sauer K, Zuniga EI. Cell-intrinsic transforming growth factor-beta signaling mediates virus-specific CD8+ T cell deletion and viral persistence in vivo. *Immunity* 2009; **31**: 145-157 [PMID: 19604493 DOI: 10.1016/j.immuni.2009.06.015]

95 **Opferman JT**, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature* 2003; **426**: 671-676 [PMID: 14668867 DOI: 10.1038/nature02067]

96 **Holz LE**, Bowen DG, Bertolino P. Mechanisms of T cell death in the liver: to Bim or not to Bim? *Dig Dis* 2010; **28**: 14-24 [PMID: 20460886 DOI: 10.1159/000282060]

97 **Radziewicz H**, Ibegbu CC, Hon H, Osborn MK, Obideen K, Wehbi M, Freeman GJ, Lennox JL, Workowski KA, Hanson HL, Grakoui A. Impaired hepatitis C virus (HCV)-specific effector CD8+ T cells undergo massive apoptosis in the peripheral blood during acute HCV infection and in the liver during the chronic phase of infection. *J Virol* 2008; **82**: 9808-9822 [PMID: 18667503 DOI: 10.1128/JVI.01075-08]

98 **Jamil S**, Wang SW, Bondy L, Mojtabavi S, Duronio V. Prevention of cytokine withdrawal-induced apoptosis by Mcl-1 requires interaction between Mcl-1 and Bim. *Biochem Cell Biol* 2010; **88**: 809-818 [PMID: 20921992 DOI: 10.1139/O10-004]

99 **Larrubia JR**, Lokhande MU, García-Garzón S, Miquel J, González-Praetorius A, Parra-Cid T, Sanz-de-Villalobos E. Persistent hepatitis C virus (HCV) infection impairs HCV-specific cytotoxic T cell reactivity through Mcl-1/Bim imbalance due to CD127 down-regulation. *J Viral Hepat* 2013; **20**: 85-94 [PMID: 23301543 DOI: 10.1111/j.1365-2893.2012.01618.x]

100 **Dzhagalov I**, Dunkle A, He YW. The anti-apoptotic Bcl-2 family member Mcl-1 promotes T lymphocyte survival at multiple stages. *J Immunol* 2008; **181**: 521-528 [PMID: 18566418]

101 **Pellegrini M**, Calzascia T, Toe JG, Preston SP, Lin AE, Elford AR, Shahinian A, Lang PA, Lang KS, Morre M, Assouline B, Lahl K, Sparwasser T, Tedder TF, Paik JH, DePinho RA, Basta S, Ohashi PS, Mak TW. IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. *Cell* 2011; **144**: 601-613 [PMID: 21295337 DOI: 10.1016/j.cell.2011.01.011]

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