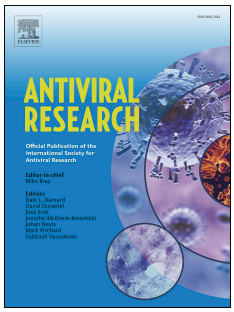

Metabolic regulation of the HBV-specific T cell function

Valeria Barili, Carolina Boni, Marzia Rossi, Andrea Vecchi, Alessandra Zecca, Amalia Penna, Gabriele Missale, Carlo Ferrari, Paola Fisicaro

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Metabolic regulation of the HBV-specific T cell function.

Valeria Barili[1,2]ǂ, Carolina Boni[2]ǂ, Marzia Rossi[1,2], Andrea Vecchi[2], Alessandra Zecca[2], Amalia Penna[2], Gabriele Missale[1, 2], Carlo Ferrari[1, 2]***,** Paola Fisicaro[1,2].

Affiliations

[1]Department of Medicine and Surgery, University of Parma, Parma, Italy.

[2]Laboratory of Viral Immunopathology, Unit of Infectious Diseases and Hepatology, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy.

ǂ These authors contributed equally to this work.

***Corresponding author**:

Prof. Carlo Ferrari

Laboratory of Viral Immunopathology, Unit of Infectious Diseases and Hepatology, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci 14, 43126 Parma, Italy.

Department of Medicine and Surgery, University of Parma, Parma, Italy.

E-mail: carlo.ferrari@unipr.it

Phone: +39-0521-702762; Fax: +39-0521-703857

Highlights

- HBV-specific CD8+ T cell dysfunction is a key determinant of chronic HBV persistence.
- HBV-specific CD8+ T cells express signaling, metabolic and transcriptional defects underlying inefficient antiviral functions.
- Targeting metabolic deregulations can restore *in vitro* efficient HBV-specific CD8+ T cell responses in chronic HBV infection.

Abstract

Chronically HBV infected subjects are more than 260 million worldwide; cirrhosis and liver cancer represent possible outcomes which affect around 700,000 patients per year. Both innate and adaptive immune responses are necessary for viral control and both have been shown to be defective in chronic patients. Metabolic remodeling is an essential process in T cell biology, particularly for T cell activation, differentiation and survival. Cellular metabolism relies on the conversion of nutrients into energy to support intracellular processes, and to generate fundamental intermediate components for cell proliferation and growth. Adaptive immune responses are the central mechanisms for the resolution of primary human infections leading to the activation of pathogen-specific B and T cell functions. In chronic HBV infection the anti-viral immune response fails to contain the virus and leads to persistent hepatic tissue damage which may finally result in liver cirrhosis and cancer. This T cell failure is associated with metabolic alterations suggesting that control of nutrient uptake and intracellular utilization as well as correct regulation of intracellular metabolic pathways are strategic for T cell differentiation during persistent chronic infections. This review will discuss some of the main features of the T cell metabolic processes which are relevant to the generation of an efficient antiviral response, with specific focus on their clinical relevance in chronic HBV infection in the perspective of possible strategies to correct deregulated metabolic pathways underlying T cell dysfunction of chronic HBV patients.

Keywords

Hepatitis B virus, chronic HBV infection, adaptive immunity, CD8+ T cell metabolism, CD8+ T cell exhaustion, metabolic restoration.

1. METABOLIC CHANGES IN T CELL DIFFERENTIATION

T cell energy requirements and substrate utilization are key elements in the metabolic reprogramming which is fundamental for adequate T cell differentiation and activation (Bantug et al., 2018; Buck et al., 2015, 2016; Chang and Pearce, 2016; Finlay and Cantrell, 2011; Klein Geltink et al., 2018; MacIver et al., 2013; Man and Kallies, 2015; O'Sullivan and Pearce, 2015; Pearce et al., 2013). Unraveling the cellular metabolic processes in the different phases of T cell development should clarify the deregulated metabolic reprogramming underlying T cell exhaustion in chronic infections. This should allow to acquire additional information helpful for the design of novel immune-modulatory strategies to cure infection.

1.1 Metabolic shift in functional effector T cell development. During primary infection, TCR recognition of peptide-MHC complexes triggers T cells to proliferate and to differentiate into functional effectors by transcriptional, epigenetic and metabolic reorganization (Ganeshan and Chawla, 2014; Pearce et al., 2013; Zuniga et al., 2015). To do this, peripheral naïve T cells increase glucose uptake to fuel mitochondrial functions and generate ATP (Klein Geltink et al., 2018; MacIver et al., 2013). Generally, ATP is produced by two main metabolic processes known as glycolysis and oxidative phosphorylation (OXPHOS) (Klein Geltink et al., 2018; MacIver et al., 2013; Pearce et al., 2013). Glucose is imported in T cells by the constitutive glucose transporter 1 (GLUT1) and transformed in pyruvate through the cytoplasmic glycolytic system involving the activation of different transcriptional and posttranslational factors (Jacobs et al., 2008; Klein Geltink et al., 2018). Hereafter, pyruvate is metabolized to lactate which is either exported from the cell also in presence of a high oxygen concentration (phenomenon known as Warburg effect or aerobic glycolysis) or transported into the mitochondria, as substrate of the tricarboxylic acid (TCA) cycle for the final generation of electrons which are needed for ATP production in the electron transport chain (ETC) and OXPHOS (Fig. 1) (Klein Geltink et al., 2018; MacIver et al., 2013; Pearce et al., 2013). Glycolysis is not only important to generate ATP but also to provide metabolic intermediates for biosynthetic pathways, needed to support rapidly dividing cells, such as effector T cells (Chapman et al., 2020; Man and Kallies, 2015). Conversely, the TCA cycle oxidizes metabolites derived

also from amino acids and fatty acids (Chapman et al., 2020; Man and Kallies, 2015). Fatty acid entrance into mitochondria is regulated by carnitine palmitoyltransferase 1a (Cpt1a) (Klein Geltink et al., 2018; Lochner et al., 2015; Mehta et al., 2017; Raud et al., 2018). In the mitochondrial matrix, fatty acid oxidation (FAO) cleaves free fatty acids with generation of acetyl-CoA, which is a substrate of the TCA cycle (Lochner et al., 2015; Mehta et al., 2017; Pearce et al., 2013; Raud et al., 2018). Another key intermediate of the TCA cycle is α-ketoglutarate, which derives from glutamine oxidation. Increased expression of both glutaminase and glutamine transporter (CD98 or ASCT2) are induced by T cell activation; glutaminase catabolizes glutamine in glutamate, which is then metabolized in the mitochondrial matrix to α-ketoglutarate which can either feed the Krebs cycle (Chapman et al., 2020; Klein Geltink et al., 2018; MacIver et al., 2013; Pearce et al., 2013; Sinclair et al., 2013) or act as substrate for the synthesis of acetyl coenzyme A, a precursor for fatty-acid synthesis, protein acetylation and chromatin structure regulation (Chisolm and Weinmann, 2018). Indeed, mitochondrial metabolites can control gene expression profiles through histone modifications (Chisolm and Weinmann, 2018). The most important mito-nuclear signaling mediator is acetyl-CoA, which is mandatory for histone acetylation associated with open and highly acetylated euchromatin, supporting active gene expression, while α-ketoglutarate (αKG) and succinate represent substrates for histone de-methylating enzymes (Chisolm and Weinmann, 2018; Mehta et al., 2017). Moreover, under high proliferation conditions, poly(ADP-ribose) polymerases (PARPs), that need NAD+ as donor of ADP-ribose, are highly active to protect T cells from genomic instability (Wang and Green, 2012). Increase in cytosolic NAD+ and citrate, the precursor of acetyl-CoA, which is induced by TCR-triggering, is thus crucial to drive T cell activation through the epigenetic regulation of specific cytokine-encoding genes (Mehta et al., 2017; Wang and Green, 2012).

Pathogen recognition with TCR activation leads also to a remarkable boost in the expression of L-amino acid transporter complexes in CD8+ T cells (Carr et al., 2010; Pearce et al., 2013; Sinclair et al., 2013; Zhang and Romero, 2018; Zhang and Bevan, 2011). This is essential to increase protein synthesis, in

order to support the replicative boost, the secretion of cytokines and chemokines (Carr et al., 2010; Ramsay and Cantrell, 2015; Zhang and Romero, 2018; Zhang and Bevan, 2011).

While quiescent T cells require low ATP levels which come from the oxidation of glucose-derived pyruvate, lipids and amino acids, TCR-mediated T cell activation requires instead increase in aerobic glycolysis with maximal production of lactate, glutamine catabolism in the mitochondria and decrease in lipid oxidation (in favor of lipid synthesis) to sustain the biosynthetic demands related to high proliferative rates (Frauwirth et al., 2002; MacIver et al., 2013; Pearce et al., 2013; Wang and Green, 2012).

Also mitochondria undergo morphological changes after T cell activation. In particular, effector CD8+ T cells undergo mitochondria fragmentation by a process known as fission (Buck et al., 2017, 2016; Klein Geltink et al., 2018). When fission is countered by pharmacological induction of mitochondrial fusion, effector T cells reduce glycolysis, and thereby activation, and acquire a T cell memory-like phenotype (Buck et al., 2016). In addition to these changes, mitochondrial biogenesis is also induced after T cell activation and leads to increased mitochondrial mass, which is mediated by the peroxisome proliferator-activated receptor γ co-activator 1α PGC1α (Buck et al., 2017; Klein Geltink et al., 2018; Ron-Harel et al., 2016).

This engagement of biosynthetic systems to produce proteins, nucleic acids, lipids and carbohydrates and other ‘building blocks’ occurs in the first 24 hours after TCR-triggering, before the first cell division (Chapman et al., 2020; Klein Geltink et al., 2018; Pearce et al., 2013; Pollizzi and Powell, 2014).

After the proliferation start, specific transcriptional signatures are activated, leading to definitive effector function maturation (Kaech and Cui, 2012; Klein Geltink et al., 2018; Man and Kallies, 2015; Rao et al., 2010; Tan et al., 2017; R. Wang et al., 2011). The aerobic oxidative metabolism distinctive of T cell activation, is sustained by the mTORC1 (rapamycin complex 1 target)-CD28 mediated signaling pathway and by the transcription factor c-Myc, which promote glucose and amino acid uptake by increasing the expression of GLUT1 and CD98 (large neutral amino acids transporter) (Carr et al., 2010; Esensten et al.,

2016; Frauwirth et al., 2002; Jacobs et al., 2008; Jones and Pearce, 2017; Klein Geltink et al., 2018; Pollizzi et al., 2015; Pollizzi and Powell, 2014; Sinclair et al., 2013; Tan et al., 2017; R. Wang et al., 2011). This glycolysis switch on is mandatory for cytokine production in effector T cells as indicated by cytokine inhibition when glycolysis is blocked. In case of aerobic glycolysis inhibition a posttranscriptional regulation of IFN-γ and IL-2 secretion occurs through the binding of the glycolysis enzyme GAPDH to the cytokine mRNA with reduction of protein translation. Instead, when glycolysis is activated, GAPDH is engaged in its metabolic function in the glycolytic pathway without inhibitory effect on cytokine production (Chang et al., 2013).

In addition, IL-2 released early after TCR engagement promotes further activation of AKT1 and mTORC1 downstream effector molecules through TCR-induced and -sustained expression of the IL‑2Rα (CD25), thereby funneling signals towards glucose and amino acid uptake and towards the expression of genes involved in protein and lipid biosynthesis (Finlay et al., 2012; Kalia et al., 2010; Man and Kallies, 2015; Pipkin et al., 2010).

1.2 Memory development. After pathogen clearance, the majority of effector T cells die and long-lived memory T cells are generated by reverting their anabolic metabolism to a mitochondrial lipid oxidation-based catabolism, with predominant OXPHOS energy production (Buck et al., 2017; Klein Geltink et al., 2018; Mehta et al., 2017; Mills et al., 2017; O'Sullivan et al., 2014; Pearce et al., 2013, 2009; van der Windt et al., 2012). Thus, high levels of glycolytic activity are a key feature of effector T cells; in contrast, an increased OXPHOS activity is mandatory for T cell memory generation. Indeed, blocking CD8+ T cell activation with the glycolysis inhibitor 2-DG (2-Deoxy-D-glucose) leads to T cell memory generation, while overexpression of glycolytic enzymes (e.g. phosphoglycerate mutase) abrogates T cell differentiation towards memory (Mehta et al., 2017; Yin et al., 2015).

The switch to an oxidative metabolism in memory T cells is associated with an increased mitochondrial biogenesis which leads to a greater mitochondrial mass and spare respiratory capacity (Man and Kallies,

2015; Pearce et al., 2013; Ron-Harel et al., 2016; van der Windt et al., 2012). Surviving lymphocytes activate AMPK which can express a number of activities (Adams et al., 2016; Blagih et al., 2015, 2012; Man and Kallies, 2015; Mayer et al., 2008; Mehta et al., 2017; Pollizzi and Powell, 2014; Rolf et al., 2013). AMPK phosphorylates and inactivates acetyl-CoA carboxylase 1 (ACC1) and the lipogenic transcription factor sterol regulatory element-binding protein 1 (SREBP1) (Blagih et al., 2015; Hardie, 2011a; Lee et al., 2014; Ma et al., 2017; Pollizzi and Powell, 2014). This ultimately results in a vigorous increase in mitochondrial fatty acid oxidation because these factors regulate the first step of de novo lipid synthesis, including the activity of the mitochondrial fatty acid transporter CPT1a (Blagih et al., 2015, 2012; Ma et al., 2017; Pearce et al., 2009). AMPK promotes also the transcriptional activity of PGC1α, which regulates positively mitochondrial biogenesis (Balmer and Hess, 2016; Blagih et al., 2015; Ma et al., 2017; Reznick et al., 2007). Furthermore, AMPK can induce the activation of the catabolic autophagy system by phosphorylating the ULK1 complex (serine/threonine protein kinase Unc-51-like kinase 1) (Egan et al., 2011; Hardie, 2011b; Kim et al., 2011; Laker et al., 2017; Löffler et al., 2011; Tamargo-Gómez and Mariño, 2018), necessary for autophagy initiation, which is extensively implicated in T cell survival and differentiation (Bronietzki et al., 2015; Levine and Deretic, 2007; Ma et al., 2013; Münz, 2009; Pua et al., 2009; Puleston et al., 2014; Schlie et al., 2015; Xu et al., 2014). Indeed, CD8+ T cells lacking the autophagosome protein ATG7, display cell-intrinsic defects precluding differentiation into long-term memory cells (Pua et al., 2009; Xu et al., 2014). AMPK can also directly regulate the degradation of disrupted mitochondria by augmenting mitochondrial fission through the phosphorylation of the mitochondrial outer-membrane protein MFF (mitochondrial fission factor). Phosphorylated MFF can recruit cytoplasmic DRP1 (dynamin 1 like protein) to the mitochondrial outer membrane, thus augmenting mitochondrial fission and increasing mitophagy of the deteriorated mitochondria (Tamargo-Gómez and Mariño, 2018).

1.3 Metabolic alterations in T cell exhaustion. It is well known that a hallmark of T cell exhaustion is PD-1 and other checkpoint inhibitor molecules overexpression (Blackburn et al., 2009; Wherry et al., 2007; Wherry and Kurachi, 2015). PD-1, and to a lesser extent also CTLA-4 engagement, can inhibit glycolysis, thus suggesting a role for inhibitory receptor signaling in the exhausted T cell metabolism (Bengsch et al., 2016; Chang et al., 2015; Parry et al., 2005; Patsoukis et al., 2015). In the LCMV model of infection, a down-regulation of glycolysis and oxidative phosphorylation associated with mitochondrial depolarization are typical of the early stages of exhaustion (Bengsch et al., 2016). These metabolic alterations are more evident in the $PD1^{hi}$ than in the $PD1^{int}$ subsets of exhausted CD8+ T cells and are maintained also during chronic infection in late exhausted T cells (Blackburn et al., 2008). PD-1 can attenuate downstream TCR signaling through its intracellular tail containing an immunotyrosine inhibitory motif (ITIM) and an immunotyrosine switch motif (ITSM) that recruit phosphatases, such as SHP-2, and mediate the dephosphorylation of key TCR signal transducers (Chemnitz et al., 2004; Hui et al., 2017; Okazaki et al., 2013; Riley, 2009; Sheppard et al., 2004). The PD-1/PD-L1 binding results in the generation of microclusters around the TCR complex (Yokosuka et al., 2012) with inhibition of proximal TCR mediators, such as PI3K and Akt/mTOR (Honda et al., 2014; Okazaki et al., 2013).

PD-1 ligation leads to a profound metabolic reprogramming towards a memory-like phenotype by blocking glycolysis and glutamine activity, reducing mTOR-mediated expression of GLUT1 and glutamine transporters, and activating mitochondrial lipid metabolism (FAO) through induction of the fatty acid transporter CPT1a to support the utilization of fatty acids as an energy source (Fig. 2) (Bengsch et al., 2016; Chang et al., 2015; Keir et al., 2008; Parry et al., 2005; Patsoukis et al., 2015). This PD-1-mediated metabolic shift from an anabolic and glycolytic towards an oxidative and mitochondrial metabolism was observed early during persistent murine chronic infection *in vivo* (Bengsch et al., 2016). In this scenario, the deterioration of the global T cell function may be due to the generation of an oxidative environment, induced by increased beta-oxidation in a setting of O_2 deficiency such as in the hypoxic liver, leading to ROS accumulation as observed in HBV-specific CD8+ T cells during chronic

infection (Fisicaro et al., 2017b). In line with this, T cell activation followed by PD-1 ligation caused depletion of the cellular antioxidant glutathione (GSH) consistent with ROS increase and subsequent oxidation-induced cellular damage (Patsoukis et al., 2015). Indeed, depending on the extent of accumulation, mitochondrial reactive oxygen species (mROS) can contribute to T cell activation or can have a detrimental effect on cell viability (Mak et al., 2017; Mehta et al., 2017; Sena et al., 2013; Siska et al., 2016). High mROS levels can lead to increased disrupted mitochondria, causing a reduction in proliferative capacity associated with apoptosis induction (Mehta et al., 2017).

Another key feature of exhausted T cells during persistent infection is the upregulation of the thymocyte selection-associated high mobility group box protein (TOX) expression (Alfei et al., 2019; Bordon, 2019; Khan et al., 2019; Sekine et al., 2020; Yao et al., 2019). TOX is known to be necessary to develop progenitor-like CD8+ T cells distinct from memory precursor cells originating in acute infection, even before the peak of the T cell response. In chronic infection it is more expressed in the terminally exhausted antigen-specific CD8+ T cells with an epigenome distinct from that of the progenitor-like cells (Alfei et al., 2019; Khan et al., 2019; Yao et al., 2019). Interestingly, beyond its positive role in the maintenance of antigen-specific CD8+ T cells during persistent infection, TOX is also directly involved in CD8+ T cell metabolic gene regulation. Indeed, TOX upregulation leads to increased expression of genes involved in the hypoxia responses and to a down-modulation of genes regulating oxidative phosphorylation, mTOR signaling, IFN-α response and DNA repair (Yao et al., 2019).

2. OXYGEN LEVEL EFFECTS ON T CELL METABOLISM

2.1 Hypoxic environment. Further important aspects in the regulation of T cell metabolism rely on the oxygen tension levels in specific tissue microenvironments. Most cellular processes are strictly dependent upon oxygen availability and different tissues and cells show different susceptibility thresholds to hypoxia (Choudhry and Harris, 2018; Lee et al., 2020; Majmundar et al., 2010; Palazon et al., 2014; Phan and Goldrath, 2015). To avoid possible severe effects caused by oxygen deprivation, mammals have

developed a tight regulatory system to deal with hypoxic conditions. Hypoxic responses generally start at 1% O_2 ($PO_2 \leq 1$ kPa or $\leq$ 7–10 mm Hg). In order to appreciate the range of oxygen levels in different body compartments, we must consider that maximal O_2 levels (13% O_2 with 13 kPa/98 mm Hg) are reached in pulmonary vessels after oxygen inspiration and decline to 5% O_2 in the tissue venous blood, to 3-5% in the interstitial tissue spaces and to 1% in intracellular compartments (Choudhry and Harris, 2018; Majmundar et al., 2010; Palazon et al., 2014; Wilson et al., 2014).

Hypoxia can be present in physiologic conditions in healthy individuals in a range of tissues, including also specific areas of the liver. Oxygen tension can dramatically decrease in pathological conditions of tissue inflammation and necrosis, as in solid tumors and infections, where increased interstitial edema can impair oxygen transport to the cells, elevated temperature can induce higher O_2 consumption and infiltration and accumulation of metabolically active immune cells can rapidly use local oxygen (Chen and Lou, 2017; Dimeloe et al., 2016; Gropper et al., 2017; Ju et al., 2016; Lee et al., 2019; Nath and Szabo, 2012; Rosmorduc and Housset, 2010; Wilson et al., 2014).

Since T cells are continuously trafficking from lymphoid organs to patrol peripheral tissues for prompt intervention at infected and inflamed sites, they must be prepared to sense and adapt their metabolism to the continuous changes in different microenvironments, even under homeostatic conditions. T cell adaptation to low O_2 levels is primarily determined by hypoxia inducible factors (HIFs), which trigger the metabolic reprogramming needed for adequate supply of energy and production of nutrients/intermediates for cell growth and division (Fig. 2) (McNamee et al., 2013; Phan and Goldrath, 2015; Sormendi and Wielockx, 2016). HIFs are heterodimeric transcription factors, known as the master controllers of the hypoxic response, involved in transcriptional reprogramming in all cells and tissues. HIFs are composed of an oxygen-sensitive a-subunit (HIF-1a, HIF-2a or HIF-3a) and of a constitutively expressed b-subunit (HIF-1b, HIF-2b, and HIF-3b). Under normal oxygen conditions (normoxia), HIFα undergoes hydroxylation, by oxygen-dependent prolyl hydroxylase domain enzymes (PHD1, PHD2 and PHD3; T cells constitutively express PHD2) as well as by asparaginyl hydroxylase enzyme FIH (Factor Inhibiting

HIF). Then, after binding to the Von Hippel-Lindau E3 ubiquitin ligase complex, HIFa undergoes ubiquitination and degradation (Lee et al., 2020).

When T cells infiltrate a hypoxic tissue (~1% O_2), the hydroxylation enzymes, which require oxygen as co-factor, become unable to mediate HIFα degradation (McNamee et al., 2013). Following stabilization, HIFα dimerizes with HIFβ, translocates in the nucleus and binds to the hypoxia response elements (HRE) within a variety of HIF target genes leading to their activation (Fig. 2) (Lee et al., 2020; McNamee et al., 2013).

Enhanced HIFα expression can also be induced in the presence of oxygen by abrogation of ubiquitin-mediated HIF degradation and upregulation of *Hif1a* gene transcription in response to stimuli able to trigger T cell receptor (TCR), Toll-like receptor (TLR) and co-stimulation signaling pathways leading to mTOR activation (Doedens et al., 2013; Phan and Goldrath, 2015).

While HIFα activity seems to be unnecessary in the first 24 h of activation for the initial transition to a glycolytic metabolism, HIF-1α may be involved in sustaining glycolytic metabolism during the T cell response (Doedens et al., 2013; Phan and Goldrath, 2015).

In CD8+ T cells HIF-1 activity drives the expression of a number of genes necessary for the shift to a glycolysis-oriented metabolism. Since activated T cells have increased energetic demands to support proliferation, which are met by a metabolic reprogramming to aerobic glycolysis and glutamine catabolism, TCR triggering activates a signaling cascade which finally ends with the activation of the transcription factor c-Myc and HIF-1α. Thus, HIFs not only control the transcriptional program for T cell adaptation to reduced oxygen levels but also contribute to the T cell metabolic reprogramming associated with T cell activation in normoxic conditions. Specifically, HIFs enhance glucose uptake through upregulation of the gene encoding the glucose transporter 1 (GLUT1) and decrease the mitochondrial oxygen consumption through the activation of genes coding for three main metabolic targets, such as lactate dehydrogenase A (LDHa), pyruvate dehydrogenase kinase 1 (PDK1) and hexokinase (HK) (Fig. 2) (Cretenet et al., 2016; Dimeloe et al., 2016; Phan and Goldrath, 2015; Xu et al., 2016). By increasing

LDH expression, the pyruvate metabolism to lactate is strongly induced. PDK1 inactivates the mitochondrial pyruvate dehydrogenase complex which usually converts glycolysis-derived pyruvate to acetyl-coA, thus blocking pyruvate oxidation in the mitochondrial Krebs cycle. HK is a key glycolysis enzyme which generates the essential intermediate glucose-6-phosphate through glucose phosphorylation (Kim et al., 2006; Phan and Goldrath, 2015). This leads to a reduced availability of pyruvate for mitochondrial fueling and to regeneration of NAD+ which is needed to support glycolysis and DNA repair mechanisms, both essential for successful progression of the early phase T cell proliferation boost (Man and Kallies, 2015). Thus, all these HIF effects eventually translate into a metabolic switch from oxygen-consuming pathways to oxygen-independent glycolytic ATP production (Phan and Goldrath, 2015).

This metabolic reprogramming underlies a series of T cell functional changes observed *in vitro* at physiologic O_2 levels which have more extensively been characterized in models of cancer pathology (Chouaib et al., 2017; Noman et al., 2015; Petrova et al., 2018). These include reduced proliferation following TCR engagement, altered cytokine production with increase in IL-17 production and Th17 differentiation, through transcriptional activation of the retinoic acid-related orphan receptor γt (RORγt), the key transcription factor for differentiation of Th17 cells (Fig. 2) (Dang et al., 2011; Shi et al., 2011). Consistent with the HIF involvement in metabolic shift towards glycolysis, HIF-1α is highly expressed by Th17 cells, which preferentially use glycolysis to meet their energy requirements. Instead, HIFs have been reported to have a negative role in the differentiation of Treg cells, which rely preferentially on OXPHOS and lipid oxidation for their energetic demands (Dang et al., 2011; Shi et al., 2011). In contrast with this conclusion, however, some studies reported Treg cell differentiation through direct transcriptional induction of FoxP3 by hypoxia and HIF (Ben-Shoshan et al., 2008; Clambey et al., 2012).

In hypoxic conditions CD8+ T cells increase their lytic activity, with the upregulation of cytotoxic proteins, such as granzyme B, TCR and adhesion molecules, but decrease their proliferative and

differentiation capacities (Fig.2) (Caldwell et al., 2001; de silly et al., 2015; Nakagawa et al., 2015; Vuillefroy de Silly et al., 2016).

Moreover, hypoxia directly increases PD-L1 expression because HIF-1α is a major regulator of PD-L1 mRNA; this PD-L1 upregulation may thus favor T cell dysfunction by triggering the inhibitory PD-1 signaling (Barsoum et al., 2014; Fisicaro et al., 2020, 2018; Labiano et al., 2015; Noman et al., 2014; Wen et al., 2020). Other HIF1α transcriptional targets are the ATP metabolizing enzymes CD39 and CD73. They promote the generation of the immune-suppressive metabolite adenosine (5'-AMP), which inhibits T and NK cell cytotoxicity within liver tumors favoring accumulation and activation of MDSCs and Treg cells (Chiu et al., 2017; Ohta, 2016; Sitkovsky and Lukashev, 2005).

2.2 The liver microenvironment. The liver has a peculiar anatomical structure with a gradient of oxygen tension from the portal venules and the hepatic arterioles, which contain oxygenated blood entering the sinusoids where oxygen is consumed (principally by the hepatocytes), to the central vein, containing oxygen depleted blood. O_2 concentrations range from approximately 12% surrounding the portal vein to 1% in the proximity of the central vein. Although the healthy liver environment is not severely hypoxic, modest O_2 changes, such as during viral hepatitis, metabolic disorders, steatohepatitis and inflammation, can induce a hypoxic response with HIFs activation. Notably, both hepatitis B and C viruses are able to induce a hypoxic-like microenvironment through HIF-1α protein stabilization (Wilson et al., 2014).

Interestingly, specific populations of memory T (T_{RM}) cells have adapted to survive in individual organ microenvironments and persist after pathogen clearance in peripheral tissues without reentering the circulation (Kim et al., 2020; Maini and Burton, 2019; Pallett et al., 2017). Similar CD8+ T cell subsets are enriched within the liver where they express distinctive phenotypes characterized by T-bet^{low} Eomes^{low} Blimp-1^{high} and by hepatic homing markers, such as CD69+ CD103+ CXCR6+ CXCR3+ (Pallett et al., 2017). This functional memory population selectively present within the liver is highly represented in chronic HBV patients with low viral load and partial immune control. Almost all tissue-resident memory

T cells in HBV-infected livers express PD-1 (more than 90%) (Fisicaro et al., 2010) and can display a specific noncytolytic response against multiple HBV proteins, with high autocrine IL-2 expression and low granzyme B production. To maintain mitochondrial fitness they express high autophagy levels to target damaged, depolarized mitochondria to the lysosome compartment for their removal. T cells with the highest level of autophagy are those that express the tissue-retention markers CD69+ and CD103+ and express better cytolytic and proliferation activity. Efficient autophagy, therefore, is likely required to allow T cells to respond to stimulation and retain effector functions (Swadling et al., 2020).

In addition to metabolic reprogramming induced by liver hypoxia, other mechanisms can modulate T cell metabolism in the liver microenvironment. Among these, arginine depletion caused by the increased expression of the arginine metabolizing enzyme arginase I released from liver infiltrating myeloid-derived suppressor cells and from damaged liver cells in chronically infected livers, can downregulate the CD3-ζ chain with inhibition of T cell proliferation by T cell arrest in G0/G1 (Fig. 3) (Fisicaro et al., 2020; Gabrilovich and Nagaraj, 2009; Munder et al., 2006; Weston et al., 2019; Zeng et al., 2019). A similar effect can be induced by L-tryptophane-degrading enzymes (TDO - TRP 2,3-dioxygenase - or IDO - indoleamine 2,3 dioxygenase) which can cause tryptophane depletion and production of the immunoregulatory metabolites L-kynurenines. While TDO is constitutively expressed in the liver, conversely, indolamine 2,3-dioxegenase 1 and 2 (IDO-1 and IDO-2) are expressed by liver-infiltrating immune cells (myeloid cells, such as monocyte/macrophages and dendritic cells) and potently activated by pro-inflammatory cytokines, such as IFN-γ or TNF-α and by superoxide anions (O_2^-) (Fig. 3) (Fisicaro et al., 2020; Heymann and Tacke, 2016; Mellor and Munn, 2004; Van der Leek et al., 2017). The local kynurenine/tryptophane balance can affect metabolism and signaling pathways of immune cells. Indeed, increased kynurenine production and tryptophan depletion induced by inflammation activate the aryl hydrocarbon receptor (AhR) transcription factor, which governs expression and secretion of IL-10 and TGF-b, thereby creating a suppressive microenvironment favorable to Treg cell generation (Mezrich et al., 2010; Sorgdrager et al., 2019). Low tryptophane concentrations in the presence of kyneurines in the

culture medium, was observed to induce the down-regulation of the TCR ζ chain, with impairment of both proliferation and cytokine secretion by CD8+ T cells (Fallarino et al., 2006), up to T cell apoptosis (Fallarino et al., 2002) associated with the emergence of IL-10 and TGF-β producing CD4+ Treg cells (Fallarino et al., 2006; Travis and Sheppard, 2014).

Tryptophane and L-arginine deprivation can trigger a compensatory upregulation of system L transporters in CHB patients, as suggested by the percentage of CD98 positive HBV-specific CD8+ T cells detected within the liver (Pallett et al., 2015). This can enhance T cell uptake of essential amino acids, like phenylalanine and leucine, which can compensate arginine and tryptophane depletion in the regulation of T cell effector function through the mTOR kinase.

3. CD8+ T CELLS IN CHRONIC HBV INFECTION.

The hepatitis B virus (HBV) is a double-stranded DNA virus with a specific liver tropism. The liver damage during HBV infection is primarily the result of the host immune response, which causes the destruction of the infected liver cells, as a protective mechanism to eliminate the virus localized intracellularly. The type of virus/host's immune system interplay is responsible for the fate of infection towards control or chronic persistence of the virus; in particular, the adaptive cell-mediated response turns out to play a key role in determining whether infection will be self-limited or chronic (Bertoletti and Ferrari, 2016; Guidotti and Chisari, 2006; Rehermann, 2013; Rehermann and Nascimbeni, 2005). Among virus-specific T cells, the CD8+ T cell population is contracted in number and greatly impaired in function when the infection persists and liver inflammation becomes chronic. This functional impairment involves both antigen-specific CD8+ T cell cytolytic activity, which is mostly mediated by perforins and granzyme B, and non-cytolytic functions, which can eliminate intracellular virus by the secretion of IFNγ and TNFα without killing the infected hepatocyte (Bertoletti and Ferrari, 2016). It is becoming increasingly clear that the distinction of HBV-specific CD8+ T cell subsets is far more complex because crucial differences are associated with different degrees of terminal differentiation towards functional

effectors or dysfunctional exhausted CD8+ T cells in individual patients with a possible role for different HBV antigen specificities (Cheng et al., 2019; Hoogeveen et al., 2018; Rehermann and Thimme, 2019; Schuch et al., 2019). Patients with chronic HBV infection have reduced frequency of HBV-specific CD8+ T cells as a result of an early activation of apoptotic processes during infection; in their induction, the apoptosis-activating BCL-2-interacting mediator of cell death (BIM) molecule has been shown to play a relevant role (Fig.3) (Holz et al., 2008; Lopes et al., 2008).

The persistent exposure to high antigen load can also contribute to T cell attrition, especially within the inflamed liver, by triggering a process of functional exhaustion which can eventually lead to T cell apoptosis if the antigenic epitope recognized by the TCR is highly expressed and the TCR/peptide-MHC interaction is of high affinity (Holz et al., 2008; Schurich et al., 2011). Upregulation of co-inhibitory ligands, such as PD-L1, on different populations of parenchymal and non-parenchymal liver cells and their binding to the corresponding T cell inhibitory receptors is central to this mechanism (Fig. 3) (Feng et al., 2015; Heymann and Tacke, 2016; Horst et al., 2016; Z.-Y. Huang et al., 2017; B.-J. Wang et al., 2011).

Also peripheral and liver-resident NK cells can negatively regulate HBV-specific CD8 T cell survival by killing them and causing their depletion through the activation of NK cell lytic mechanisms sustained by NKG2D and/or TRAIL receptor/ligand pair interactions between NK cells and T cells within the chronically HBV infected liver (Fig. 3) (Boni et al., 2015; W.-C. Huang et al., 2017; Lang et al., 2012; Peppa et al., 2013; Waggoner et al., 2011).

Additional general T cell attrition mechanisms which are not HBV-specific may be operative within the liver. For example, Kupffer Cells (KCs) may have a regulatory role which relies on their inducible expression of Fas-L by IFN-γ produced by antigen-triggered T cells or by gut-derived endotoxins, which can lead to direct apoptotic elimination of activated T cells in the liver (Fig.3) (Horst et al., 2016; Muschen et al., 1999; Uchikura et al., 2004).

HBV-specific CD8+ T cells which survive these attrition mechanisms, are poorly functional in terms of cytokine production, cytotoxicity and expansion capacity as compared to memory CD8+ T cells of spontaneously resolved acute HBV patients (Bertoletti and Ferrari, 2016; Boni et al., 2007; Ferrari et al., 2017; Knolle et al., 2015; Maini and Burton, 2019; Shin et al., 2016).

Recent studies have provided some novel important insights into our understanding of the molecular and metabolic mechanisms underlying the T cell failure in the control of persistent viral infections. The discussion of these new data will be the focus of the second part of this review.

4. MOLECULAR AND METABOLIC DYSFUNCTION OF HBV-SPECIFIC CD8+ T CELLS IN CHRONIC HEPATITIS B

The HBV-specific adaptive immune response is selectively able to recognize and hit HBV by targeting the infected hepatocytes, but virus-specific T cells are deeply dysfunctional in CHB. Thus, their functional restoration represents a rational approach for novel and innovative immunotherapies and understanding the molecular and metabolic basis of T cell dysfunction is essential in this perspective.

First of all, exhausted HBV-specific CD8+ T cells express high levels of checkpoint inhibitory receptors, including PD-1, CTLA-4, TIM-3 and CD244, which are more strongly upregulated on intrahepatic HBV-specific CD8+ T cells and can affect T cell metabolism (Fig. 3) (Boni et al., 2007; Fisicaro et al., 2012, 2010; Nebbia et al., 2012; Raziorrouh et al., 2010; Schurich et al., 2011). For example, engagement of the upregulated PD-1 with its upregulated ligand within the liver can cause glycolysis inhibition. The expression of inhibitory receptors is not homogeneously distributed in the overall HBV-specific CD8+ T cell population because different ratios of more or less terminally differentiated CD8+ T cell subsets with different phenotypic and functional features can co-exist in individual infected hosts (Cheng et al., 2019; Hoogeveen et al., 2018; Schuch et al., 2019). In relation to their relative prevalence, the capacity to control infection of each individual patient is likely different as well as the capacity to respond *in vitro*

(and presumably for inference also *in vivo*) to immune modulation. This is similar to what described in chronically LCMV infected mice where the expression of the transcription factors Tbet and EOMES allows to define two differently programmed subsets. The small Tbet PD-1^{int} progenitor pool characterized by residual proliferative potential and capacity to produce cytokines is more responsive to the PD-1 pathway blockade restoration effect. Conversely, the numerically larger population of EOMES terminal progeny featured by a higher expression of PD-1 and other inhibitory receptors, displays limited proliferative capacity and little or no response to PD-1 blockade (Doering et al., 2012; McLane et al., 2019).

A phenotypic and functional HBV-specific CD8+ T cell heterogeneity is a feature of CD8+ T cells targeting different HBV proteins (Cheng et al., 2019; Hoogeveen et al., 2018; Schuch et al., 2019). Indeed, a more severe degree of T cell exhaustion with more profound functional impairment associated with up-regulation of CD38, KLRG1 and EOMES but reduction of Tbet and CD127 has been reported for virus-specific CD8+ T cells targeting polymerase epitopes compared to CD8+ T cells specific for the HBV core protein. The latter displays an enhanced proliferative capacity associated with a more protective memory-like CD127+PD1+ phenotypic profile (Hoogeveen et al., 2018; Schuch et al., 2019). However, phenotypic and functional HBV-specific CD8+ T cell heterogeneity cannot be simply explained by a different antigenic specificity because variability in subset representation can be observed also in the context of CD8+ T cell populations of single epitope specificity, such as HBV core 18-27 specific CD8+ cells (Personal communication, C. Boni, M. Rossi, A.Vecchi and C. Ferrari).

The overall exhausted CD8+ T cell population from chronically infected patients shows a distinctive transcriptional fingerprint which translates into specific metabolic and signaling abnormalities within HBV-specific CD8+ T cells (Fig. 3), which in turn represents the molecular basis of the anti-viral CD8+ T cell dysfunction. A major transcriptional deregulation in CD8+ T cells involves mitochondrial genes with enrichment in downregulated transcripts related to key mitochondrial processes, such as the electron-transport chain, the transport across mitochondrial membranes and cellular metabolism, comprising fatty

acid oxidation and amino acid metabolism. In addition, also genes coding for important components of the cellular machinery responsible for transcription and/or translation as well as heme and other Fe2+-containing cofactor biosynthesis and genes related to the mitochondrial quality-control system appear to be significantly downregulated in exhausted CD8+ T cells (Fisicaro et al., 2017b). Importantly, this wide transcriptional deregulation translates into a parallel alteration of the corresponding protein levels and mitochondrial functions. Indeed, dextramer-positive CD8+ T cells from patients with chronic infection display reduced mitochondrial polarization and mass biogenesis and show significantly higher mitochondrial ROS content, as compared to healthy controls and patients able to resolve HBV infection spontaneously.

ROS are signaling molecules which are needed for T cell activation. However, if their production exceeds the cellular anti-oxidant neutralization capacity, they can become harmful to the cell function by causing cellular damage at DNA and protein/organelles levels and possible proteostasis engulfment. This is the situation detected in HBV-specific CD8+ T cells of patients with chronic HBV infection that show accumulation of abnormal quantities of intracellular aggresomes (Fig. 3) (Fisicaro et al., 2017).

Misfolded/damaged protein digestion and recycle is primarily accomplished by the ubiquitin-proteasome system and by autophagy, which is a lysosome-mediated degradation pathway involved in aggregated protein removal and in cytoplasmic organelle turnover (Kocaturk and Gozuacik, 2018). Interestingly, also mitochondria play a role in cytosolic aggregate disassembly by the active import of proteins into the mitochondrial matrix for protease digestion (Ruan et al., 2017). In case of oxidized protein accumulation derived from excess ROS production by dysfunctional mitochondria, the proteasome and autophagy systems are expected to increase their function to avoid a pathologic accumulation of protein aggregates which can form cytotoxic complexes harmful to the T cell function (D'Amico et al., 2017; Gumeni and Trougakos, 2016; Sorrentino et al., 2017; Tsakiri et al., 2019; Widjaja et al., 2017). Excess ROS production can also lead to DNA damage and subsequent activation of cellular DNA repair mechanisms (Korovila et al., 2017). If ROS overproduction becomes persistent, as a result of a chronic T cell

stimulation by persisting HBV antigen production, all these cellular protein/DNA quality control systems can be overwhelmed becoming insufficient to guarantee a normal protein/organelle turnover and DNA repair (Fernandez-Mosquera et al., 2019). This can be ultimately deleterious for the cellular function with possible progression to cell death (Berges et al., 2009). In this condition of chronic liver inflammation, HBV-specific CD8+ T cells from chronic HBV patients show a strong downregulation of genes related to proteasome and lysosome function as well as DNA damage responses (Fisicaro et al., 2017). The former include genes coding for 26S proteasome subunits, for molecules required for autophagosome formation, such as clathrin, autophagosome maturation, such as GORASP2, autophagosome degradation, such as the TCIRG1 or ATP6V0C H+-ATPases, as well as genes coding for components of the lysosome degradation machinery, including peptidases and galactosylceramidases (Fisicaro et al., 2017). Among DNA repair transcripts, both p53-dependent (such as the ATM kinase) and p53-independent (RAD 17, 21, 23A and 23B) DNA-repair protein coding genes appear to be significantly downregulated as well as genes coding for the repair DNA polymerase POLH and for proteins with a specific role in genome-integrity defense, such as the telomere protein TERF2IP (Fisicaro et al., 2017b). This transcriptional deregulation corresponds to a real functional impairment of both proteostasis and DNA damage responses (P. Fisicaro et al., 2017) as indicated by an abnormal accumulation of intracellular aggresomes and an altered autophagosome function with impaired generation of autophagic vesicles in HBV-specific CD8+ cells (Acerbi et al., 2020 *in press*) and an impairment of some DNA repair mechanisms.

Although down-regulation is the prevalent transcriptional phenotype of exhausted T-cells, some genes appear to be upregulated and this may further amplify the T cell functional impairment, because most of these upregulated genes encode negative transcriptional regulators or otherwise repressive molecules, including for example multiple C2H2 zinc fingers and KRAB domain-containing proteins, and the histone deacetylase HDAC1 (Fig. 3) (Fisicaro et al., 2017b).

The overall picture emerging from all this new information on exhausted HBV-specific CD8+ T cells is consistent with a deep cell impairment at biosynthetic, genome safeguard and energetic/metabolic levels,

with the involvement of multiple pathways and cellular processes, centered on mitochondrial dysfunction and overwhelming ROS production. This mitochondrial dysfunction is associated with an increased expression of functional Glut1 transporter molecules upon antigenic stimulation, suggesting the functional dependency of HBV-specific CD8+ T cells on glycolysis (Fig. 3) (Schurich et al., 2016). This is supported by the finding that CD8+ T cell culture in galactose, to block glycolysis, abrogates cytokine production, showing that energy supply in exhausted HBV-specific T cells relies on glycolysis and that glycolysis cannot be compensated by other metabolic pathways (Schurich et al., 2016). Thus, the impaired effector function of HBV-specific CD8+ T cells should not be due to a lack of glucose uptake but rather to their poor capacity to use oxidative phosphorylation to support their energy requirements. This makes T cells unable to undergo metabolic reprogramming from glycolysis to oxidative phosphorylation to meet the bioenergetic demands of protective T cell memory development.

Interestingly, while high PD-1 expression in exhausted T cells is associated with a greater GLUT1 expression and low mitochondria functionality, in early activated CD4 T cells PD-1 engagement can reduce glycolysis and promote mitochondrial fatty acid oxidation (Patsoukis et al., 2015).

In fact, exhausted HBV-specific T cells depict a complex scenario which could be, at least in part, a consequence of the hypoxic milieu of the liver. In this compartment HIFs signaling can induce the transcription of Glut1 as well as of several rate-limiting glycolytic enzymes which may allow to overcome the energy imbalance due to the hypoxia-induced decline in mitochondrial activity, as suggested also by Mala Maini's group (Schurich et al., 2016).

As reported in chronic viral infections in mice, T cell exhaustion in these models is associated with an altered chromatin accessibility and the acquisition of transcriptionally repressive DNA methylation profiles involved in establishing stable gene-silencing programs which are only partially remodeled by PD-1 blockade (Ahn et al., 2016; Ghoneim et al., 2017; Khan et al., 2019; Pauken et al., 2016; Sen et al., 2016). Also in HBV specific CD8+ T cells the profound and extensive gene downregulation is associated with enhanced expression of negative transcriptional regulators, such as for example the histone

deacetylase HDAC1 (Fisicaro et al., 2017b), suggesting the establishment of extensive epigenetic changes throughout the infection lifespan raising the unsolved issue of whether metabolic/signaling corrections can allow to reacquire durable immune memory, especially if viral antigens persist.

5. STARTEGIES FOR T CELL METABOLIC RESTORATION

Novel potential immunomodulatory approaches rely on targeting different cellular components of the immune system. In particular, some strategies aimed at T cell functional restoration in CHB infection, such as cytokine-mediated immune modulation, result in a shift in T cell metabolism. For example, the treatment with the pro-inflammatory cytokine IL-12 can improve the HBV-specific CD8+ T cell function with a more powerful effect when it is combined with PD-1 blockade. IL-12 alone is effective in restoring both cytokine secretion and cytotoxic activity, reducing Bim-mediated apoptosis, due to the increased expression of the cytokine-regulatory transcription factor Tbet, along with PD-1 and Bim decline (Schurich et al., 2013). IL-12 can act on mitochondrial fitness by reducing the percentage of depolarized mitochondria and can reverse T cell dependence on glycolysis for effector functions (Schurich et al., 2016). However, systemic IL-12 administration in cancer trials was poorly tolerated; this represents a possible limitation to its use *in vivo* for the treatment of CHB patients, although a clinical trial with a DNA vaccine for CHB based on plasmids encoding viral protein sequences in association with a human IL-12 plasmid is currently ongoing (Boni et al., 2019a; Maini and Burton, 2019; Maini and Pallett, 2018; Schurich et al., 2016) . In addition, IL-12 modulation with possible restoration of HBV-specific CD8+ T cell responses may also be mediated by TLR-8 agonist GS-9688 administration, which is under evaluation in a phase II trial for CHB patients (Jo et al., 2014; Mackman et al., 2020; McGowan et al., 2016).

A recent study showed that also IL-2 treatment, known to be able to stimulate both glucose and amino acid uptake as well as the expression of genes involved in protein and lipid biosynthesis (Finlay et al., 2012; Kalia et al., 2010; Man and Kallies, 2015; Pipkin et al., 2010), can lead to a better T cell effector

differentiation of HBV-specific CD8+ T cells. Indeed, through the administration of recombinant IL-2 to HBV transgenic mice, virus-specific CD8+ T cells restored their ability to proliferate and differentiate in IFN-γ-producing cells (Bénéchet et al., 2019). These findings were confirmed in *in vitro* experiments, where virus-specific T cells from immune-tolerant HBV infected patients were rescued by IL-2 addition, reaching the mean IFN-γ production level observed in immune-active patients (Bénéchet et al., 2019). These results show the potential effectiveness of a modified IL-2 molecule (named IL-2c), which had been adapted for the reduction of toxic systemic effects and for targeting only CD8+ T cells instead of Tregs, as a new immunotherapeutic approach in chronic HBV infection (Bénéchet et al., 2019; Spolski et al., 2018).

Also the effectiveness of the checkpoint blockade approach, widely described in the context of chronic HBV infection (Boni et al., 2019a, 2007; Evans et al., 2008; Fisicaro et al., 2012; Maier et al., 2007; Maini and Burton, 2019), can be attributable to a T cell metabolic modulation. PD-1 has been shown to negatively regulate PI3K and Akt/mTOR signalings, which in activated T cells represent the main metabolic transducers for the direct engagement of glycolysis (Patsoukis et al., 2015). Thus, PD-1 ligation was observed to induce an enormous metabolic reprogramming toward a memory-like phenotype by blocking the glycolytic and glutaminic activity, reducing the mTOR-mediated expression of GLUT1 and glutamine transporters, and activating mitochondrial lipid metabolism (FAO) through the induction of the fatty acid transporter CPT1A to support the utilization of fatty acids, as an energy source (Bengsch et al., 2016).

The importance of ROS neutralization for the functional recovery of exhausted HBV-specific T cells has been reported in a study from our group. Upon exposure to mitochondria-targeted antioxidants, such as the ubiquinone-derivative MitoQ and the superoxide dismutase mimetic MitoTEMPO, CD8+ T cells from chronic HBV patients showed an increased expansion capacity and a polyfunctional cytokine restoration (Fisicaro et al., 2017b). These ROS scavenger compounds caused also the reduction in AnnexinV+ virus-specific CD8+ T cells, typically associated with apoptosis, likely due to the observed increase in

functional mitochondria and in the expression of electron transport chain mitochondrial proteins. This restoring effect has been shown also in liver-infiltrating CD8+ and CD4+ cells isolated from liver biopsy tissue of CHB patients (Fisicaro et al., 2017b).

Also the treatment with the ROS scavenger NAC was tested on dysfunctional and so-called 'suppressor' CD8+ T cells in CHB patients, intriguingly reducing IL-10 production and increasing IFN-γ in the overall CD8+ T cell population, again confirming the deleterious role of ROS in T cells from CHB patients (Mohanty et al., 2020).

In addition to mitochondria alterations, in chronic hepatitis B antiviral T lymphocytes resulted deeply impaired with several metabolic and intracellular dysfunctions, such as deregulation in protein degradation mechanisms (Acerbi et al., 2020 *in press*; Fisicaro et al., 2017b), which represent an appealing therapeutic target to cure CHB by functional T cell reconstitution. It is known that natural polyphenols, such as resveratrol and oleuropein, foster AMPK phosphorylation with sirtuin upregulation, and induce an antioxidant response on cells undergoing oxidative stress (Bruckbauer and Zemel, 2014; Dhouafli et al., 2018). In fact, *in vitro* exposure to resveratrol and oleuropein polyphenols, was able to improve both the mitochondrial function, with reduction of ROS accumulation and depolarized mitochondria, and proteostasis, with diminution of unfolded protein aggregates together with recovery of the antiviral T cell function (Acerbi et al., 2020 *in press*). This effect was further strengthened in a significant proportion of patients by the association of polyphenols with antioxidant compounds, such as MitoQ and MitoTempo (Acerbi et al., 2020 *in press*). Thus, the combination of antioxidants and natural polyphenols represents a promising strategy for chronic HBV infection which could be alternative or complementary to the checkpoint blockade, in order to restore T cell function and responsiveness to antigen stimulation and render HBV-specific T cells more sensitive to therapeutic vaccination.

6. WHERE ARE WE GOING?

Many novel therapeutic strategies are currently being proposed and some of them tested in the clinics to fulfill the need of a sustained and off-treatment suppression of HBV replication in chronically infected patients by restoring HBV-specific adaptive immune responses (Boni et al., 2019b; Fanning et al., 2019; Rehermann and Thimme, 2019; Revill et al., 2019).

As chronic HBV infection is the result of HBV-specific immune tolerance, breaking local mechanisms of immune silencing and restoring HBV-specific immune responses may eventually allow HBV clearance. The most promising therapeutic approaches to restore the T cell function include checkpoint blockade (Baumeister et al., 2016; Fisicaro et al., 2018; Maini and Burton, 2019; Nebbia et al., 2012; Pardoll, 2012; Raziorrouh et al., 2010; Wu et al., 2012), especially PD-1/PD-L1 interaction blockade (Bengsch et al., 2014; Fisicaro et al., 2012; Jacobi et al., 2019), metabolic and mitochondrial targeting (Bénéchet et al., 2019; Fisicaro et al., 2017b; Schurich et al., 2016, 2013), therapeutic vaccination (Boni et al., 2019c; Cavenaugh et al., 2011; Dembek et al., 2018; Fontaine et al., 2015; Godon et al., 2014; Kosinska et al., 2017; Li et al., 2017; Lobaina and Michel, 2017; Mahtab et al., 2018; Xu et al., 2013; Yoon et al., 2015; Zhou et al., 2017), reduction of antigen load and blockade of inhibitory pathways (Fig. 2) (Boni et al., 2019b; Fisicaro et al., 2018; Gehring and Protzer, 2019; Hoogeveen and Boonstra, 2020; Maini and Burton, 2019).

In alternative, immune therapies can be aimed at replacing dysfunctional T cells by providing the infected host with newly *in vitro* generated TCR-redirected T cells or CAR T cells (Bertoletti et al., 2017; Bohne et al., 2008; Festag et al., 2019; Gehring et al., 2011; Kah et al., 2017; Koh et al., 2018; Krebs et al., 2013; Kruse et al., 2018; Qasim et al., 2015; Tan et al., 2019). Immuno-modulatory therapies could carry the risk of going beyond immune control causing undesired hepatic damage. Therefore, the combined use of potent antiviral treatments and selective immune modulation may be the best strategy to accomplish a functional cure for HBV, without inducing severe tissue damage and disease progression.

In the perspective of new targeted therapeutic approaches, the issue of whether a metabolic recovery is actually sufficient for functional restoration of the anti-viral T cell function should be better addressed. Many different metabolic processes and signaling pathways have been reported to be deregulated in HBV-specific CD8+ cells of chronic HBV patients, but we still need to understand which ones of these potential molecular targets are more suited to accelerate a complete control of infection.

A key aspect of the immune-metabolic T cell alterations which needs to be better investigated, is to define to what extent peripheral blood T cells can recapitulate the front-line immune-surveillance mediated by the liver infiltrating and liver-resident T cells which are forced to survive and to express their function in a severely tolerogenic and hypoxic environment. A rational design of functional cure strategies by metabolic T cell modulation necessarily requires to better delineate transcriptional, metabolic and signaling features of intrahepatic HBV-specific T cells. This is mandatory for correct *in vivo* reprogramming of the T cell responses to endure the hostile liver microenvironment. In this respect, an important tool consists in the use of fine needle aspirates (FNAs) for the study of liver tissue. Although carefully conducted comparisons of FNAs and biopsies indicated that also FNAs can allow to reproduce the intrahepatic T cell compartment, the limited number of T cells that can be retrieved from individual FNAs represents an important limitation to their use for in-depth functional studies (Gill et al., 2019).

Among the molecular features underlying T cell exhaustion shared by peripheral blood T cells of different chronic viral infections (Chisolm and Weinmann, 2018), deregulation of chromatin accessibility with induction of repressive chromatin remodeling programs highlights the possible role of epigenetic drugs (Sen et al., 2016). Several epigenetic drugs are already FDA-approved for cancer treatment (Dunn and Rao, 2017; Weintraub, 2016). In HCV infection, epigenetic approaches have been successfully tested *in vitro* by targeting the histone methyltransferases, EZH2 and G9a, which were transcriptionally upregulated in exhausted T cells from HCV chronic patients, inducing restoration of CD8+ T cell cytokine production, proliferation and metabolism (Barili et al., 2020). A synergistic effect was observed

between several epigenetic inhibitors (e.g. blockade of EZH2) and checkpoint blockade treatments (such as with anti-CTLA-4 and anti-PD-1 treatment) (Goswami et al., 2018; McGoverne et al., 2020).

Finally, infected patient heterogeneity represents another issue to take into consideration in the perspective of personalized therapeutic approaches. Identification of patient subgroups with different propensity to respond to metabolic/immune modulation is compulsory and the use of artificial intelligence, to make an exhaustive combined analysis of genetic, metabolic, signaling, functional data in each individual patient will hopefully allow to identify a sensitive and specific prediction algorithm for chronic HBV infection, such as those used to drive immune-therapeutic choices in the cancer field (Huemer et al., 2020).

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FIGURE LEGENDS

Figure 1. Metabolic profiles of naïve, effector and memory CD8+ T Cells.

CD8+ T cells undergo sequential steps of metabolic reprogramming during their differentiation to meet the different energy requirements related to the naïve, effector and memory stages. Naïve CD8+ T cells (*on the left*) are metabolically quiescent with low metabolic, transcriptional and translational activities, low rates of oxygen and glucose consumption and minimal biosynthesis. Energy generation is primarily dependent on mitochondrial oxidative phosphorylation (OXPHOS). As a pro-survival factor for resting peripheral naïve T cells, IL-7 is critically important through the activation of the IL-7 receptor (IL-7R) which leads to the activation of the PI3K/Akt/mTOR signaling pathway. The IL-7 signaling allows basal regulation of glucose uptake and amino acids metabolism through cell surface trafficking of Glut1 and ASCT2, respectively. The lack of adequate extrinsic signals drives reduction in Glut1 expression resulting in glycolysis drop and energy imbalance. Accordingly, a derived elevated AMP to ATP ratio activates AMPK to sustain mitochondrial energy production and blocking anabolic mTOR activities (Jacobs et al., 2010; Rathmell et al., 2000; Tan et al., 2001).

Effector CD8+ T cells (*middle panel*), upon antigen receptor stimulation, increase glycolysis and OXPHOS which are both responsible for enhanced ATP production. Thus, glucose metabolism in activated T cells shifts from a prevalent use of oxidative phosphorylation to a strong upregulation of the glycolytic pathway. Building blocks crucial for the initial clonal expansion are produced by glycolysis and glutaminolysis. The exit from quiescence is essentially driven by downstream T cell receptor (TCR) and CD28 costimulatory signaling pathways, reinforced by IL-2 receptor activation. After TCR stimulation, mTORC1 activation and the subsequent induction of c-Myc expression promote the upregulation of i) glucose transporter 1 (GLUT1), to fuel aerobic glycolysis which is mandatory for proliferation and cytokine productions, ii) glutamine transporter (ASCT2), to fuel mitochondrial OXPHOS, iii) CD98 amino acid transporter, that mediates the uptake of large neutral amino acids, such as leucine and methionine, needed for the protein synthesis burst, which in turn is necessary for effector T

cell functions and proliferation. Moreover, engagement of fatty acids synthesis (FAS) and import (by the CD36 transporter) is required to supply intermediates for membrane synthesis during T cell activation and division) (Lochner et al., 2015).

CD8+ memory T cells (*on the right*) downregulate glycolysis and become strictly dependent on fatty acid oxidation (FAO) and mitochondrial metabolism to meet their energy requirements and support their function. Thus, effector CD8+ T cell function is primarily sustained by glycolysis, while memory T cells mainly rely on OXPHOS. The homeostasis of memory T cells is mainly sustained by IL-15 and IL-7 downstream signaling, in order to keep memory cells in a 'poised' state of quiescence (Chapman et al., 2020). During the transition of effector T cells toward memory differentiation, AMPK activity is essential to induce mitochondrial FAO by driving the activation of the mitochondrial transporter of fatty acids Cpt1a and of the mitochondrial biogenesis-regulator PGC1α (Pollizzi and Powell, 2014). AMPK is also mandatory to mount an effective secondary response to an *in vivo* infection, as observed in AMPKα1-deficient CD8+ T cells (Rolf et al., 2013).

Metabolically primed memory T cells display an increased mitochondrial mass with mitochondrial architecture preserved, primarily by autophagy-mitophagy activity which is essential to disrupt damaged mitochondria and sustain fusion of functional mitochondria resulting in higher spare respiratory capacity. Memory T cells use newly generated fatty acids to fuel FAO. Fatty acids are produced intracellularly starting from glucose which is metabolized to pyruvate and used in the TCA cycle to produce citrate which is exported out of the mitochondria to generate acetyl-CoA and then fatty acids. The latter are stored as neutral lipids in lysosomes and then mobilized by lysosomal hydrolase (LAL) (lyposomal lipolysis) to re-enter mitochondria through Cpt1a for degradation in the mitochondrial FAO, thereby engaging a 'futile' cycle of de novo lipid synthesis and subsequent lipolysis to produce fatty acids used for FAO (Lochner et al., 2015).

To mount an effective secondary response upon restimulation, memory CD8+ T cells employ ATP generated by mitochondrial FAO for an accelerated induction of glycolysis by hexokinase (HK; the first

enzyme of the glycolytic pathway) activation to phosphorylate imported glucose (Gubser et al., 2013; Van Der Windt et al., 2013). Created with BioRender.com.

Figure 2. Hypoxic microenvironment and CD8+ T cell metabolic reprogramming.

Schematic representation of the hypoxia-induced suppressive mechanisms in low O_2 microenvironment (*upper part*) and their consequences in effector CD8+ T cells (*lower part*). Specifically, Th17 and Treg differentiation are induced by transcriptional activation of key transcription factors. Hypoxia directly increases PD-L1 expression on different immune-suppressive populations, such as on M2-like macrophages and myeloid-derived suppressor cells (MDSCs), associated with up-regulation of the adenosine-producing enzymes CD39 and CD73 on T cells. CD39 overexpression leads to ATP degradation in AMP, which is digested into the immunosuppressive metabolite adenosine by CD73. Intracellularly, under normoxic conditions HIF-1α levels are primarily controlled by proteasomal degradation through ubiquitination by PHDs that are only active in the presence of O_2. Instead, under hypoxic conditions, stabilized HIF-1α moves into the nucleus and exerts direct and indirect transcriptional activation by partnering with the constitutively expressed HIF-1β protein. Activation of the intracellular HIF-1 pathway increases glucose channeling into glycolysis by up-regulating GLUT transporters and key metabolic enzymes such as lactate dehydrogenase (LDH), which drives pyruvate conversion into lactate away from mitochondria. Another HIF-1-induced key enzyme is pyruvate dehydrogenase kinase 1 (PDK1), which inhibits mitochondrial pyruvate dehydrogenase (PDH) with further prevention of pyruvate oxidation in mitochondria and subsequent decrease in mitochondrial oxidative phosphorylation (OXPHOS). HIF-1 also augments the lactate efflux transporters (MCTs) expression to remove excess lactate from the cytoplasm, resulting in an increase in extracellular acidification. Under hypoxic conditions, also glutamine metabolism is augmented, in order to stimulate fatty acid and amino acid biosynthesis for energy production. Moreover, hypoxic CTLs contain normal

numbers of cytolytic granules with more granzyme-B but proliferate more slowly. Created with BioRender.com.

Figure 3. Potential therapeutic strategies for functional T cell reconstitution in chronic HBV infection.

On the left, the different recognized mechanisms underlying CD8+ T cell exhaustion are represented as three different levels of intracellular deregulation. i) Metabolic alterations: they are mainly due to the accumulation of dysfunctional mitochondria with increased ROS production caused by an inefficient electron transport chain (ETC). This stimulates DNA damage which is also contributed by the high cell proliferative rate. Increased expression of glucose transporter 1 (Glut1) and neutral amino acid transporter (CD98) derives from a feedback mechanism due to energy requirement coupled with dysfunctional mitochondria and to amino acid deprivation caused by a suppressive microenvironment enriched in amino acids degrading enzymes (e.g. Arginase I and IDO). ii) Autophagy/proteasome impairment: it is responsible for accumulation of unfolded/oxidized enzymes and proteins and of damaged mitochondria with a defective biomolecule recycling. iii) Signaling deregulations: increased expression of co-inhibitory receptors (such as PD-1, CTLA-4, TIM-3, etc.) and reduced levels of co-stimulatory molecules (such as CD28 and CD3ζ chain) lead to downstream effector signaling activity reduction (such as the TCR-driven AKT-mTOR system). Moreover, apoptotic mechanisms are sustained by increased expression of the apoptosis-activating BCL-2-interacting mediator of cell death (BIM) molecule, by NK-induced signaling through TRAIL and NKG2D receptors and by Kupffer cell-mediated FAS/FASL interaction.

On the right, several different approaches of functional CD8+ T cell restoration are highlighted as potential immune modulatory treatments for chronic HBV infection, based either on a direct effect on intracellular effectors of T cell exhaustion (*upper boxes*), or on substitution of dysfunctional T cells (e.g. with TCR-redirected/CAR T cells) and on T cell boosting with antigen vaccination (*lower boxes*). Created with BioRender.com.

REFERENCES

Acerbi, G., Montali, I., Ferrigno, G., Barili, V., Schivazappa, S., Arianna, A., Laccabue, D., Loglio, A., Borghi, M., Marco, M., Rossi, M., Vecchi, A., Penna, A., Boni, C., Missale, G., Lampertico, P., Del Rio, D., Ferrari, C., Fisicaro, P., 2020. Functional reconstitution of HBV-specific CD8 T cells by in vitro polyphenol treatment in chronic hepatitis B. J. Hepatol. 11, S0168-8278. https://doi.org/10.1016/j.jhep.2020.10.034

Adams, W.C., Chen, Y.-H., Kratchmarov, R., Yen, B., Nish, S.A., Lin, W.-H.W., Rothman, N.J., Luchsinger, L.L., Klein, U., Busslinger, M., Rathmell, J.C., Snoeck, H.-W., Reiner, S.L., 2016. Anabolism-Associated Mitochondrial Stasis Driving Lymphocyte Differentiation over Self-Renewal. Cell Rep. 17, 3142–3152. https://doi.org/10.1016/j.celrep.2016.11.065

Ahn, E., Youngblood, B., Lee, J., Lee, J., Sarkar, S., Ahmed, R., 2016. Demethylation of the PD-1 Promoter Is Imprinted during the Effector Phase of CD8 T Cell Exhaustion. J. Virol. 90, 8934–8946. https://doi.org/10.1128/JVI.00798-16

Alfei, F., Kanev, K., Hofmann, M., Wu, M., Ghoneim, H.E., Roelli, P., Utzschneider, D.T., von Hoesslin, M., Cullen, J.G., Fan, Y., Eisenberg, V., Wohlleber, D., Steiger, K., Merkler, D., Delorenzi, M., Knolle, P.A., Cohen, C.J., Thimme, R., Youngblood, B., Zehn, D., 2019. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. Nature. https://doi.org/10.1038/s41586-019-1326-9

Balmer, M.L., Hess, C., 2016. Feeling Worn Out? PGC1α to the Rescue for Dysfunctional Mitochondria in T Cell Exhaustion. Immunity. https://doi.org/10.1016/j.immuni.2016.07.024

Bantug, G.R., Galluzzi, L., Kroemer, G., Hess, C., 2018. The spectrum of T cell metabolism in health and disease. Nat. Rev. Immunol. 18, 19–34. https://doi.org/10.1038/nri.2017.99

Barili, V., Fisicaro, P., Montanini, B., Acerbi, G., Filippi, A., Forleo, G., Romualdi, C., Ferracin, M., Guerrieri, F., Pedrazzi, G., Boni, C., Rossi, M., Vecchi, A., Penna, A., Zecca, A., Mori, C., Orlandini, A., Negri, E., Pesci, M., Massari, M., Missale, G., Levrero, M., Ottonello, S., Ferrari, C., 2020. Targeting p53 and histone methyltransferases restores exhausted CD8+ T cells in HCV infection. Nat. Commun. https://doi.org/10.1038/s41467-019-14137-7

Barsoum, I.B., Smallwood, C.A., Siemens, D.R., Graham, C.H., 2014. A Mechanism of Hypoxia-Mediated Escape from Adaptive Immunity in Cancer Cells. Cancer Res. 74, 665–674. https://doi.org/10.1158/0008-5472.CAN-13-0992

Baumeister, S.H., Freeman, G.J., Dranoff, G., Sharpe, A.H., 2016. Coinhibitory Pathways in

Immunotherapy for Cancer. Annu. Rev. Immunol. https://doi.org/10.1146/annurev-immunol-032414-112049

Ben-Shoshan, J., Maysel-Auslender, S., Mor, A., Keren, G., George, J., 2008. Hypoxia controls CD4+ CD25+ regulatory T-cell homeostasis via hypoxia-inducible factor-1α. Eur. J. Immunol. https://doi.org/10.1002/eji.200838318

Bénéchet, A.P., De Simone, G., Di Lucia, P., Cilenti, F., Barbiera, G., Le Bert, N., Fumagalli, V., Lusito, E., Moalli, F., Bianchessi, V., Andreata, F., Zordan, P., Bono, E., Giustini, L., Bonilla, W. V., Bleriot, C., Kunasegaran, K., Gonzalez-Aseguinolaza, G., Pinschewer, D.D., Kennedy, P.T.F., Naldini, L., Kuka, M., Ginhoux, F., Cantore, A., Bertoletti, A., Ostuni, R., Guidotti, L.G., Iannacone, M., 2019. Dynamics and genomic landscape of CD8+ T cells undergoing hepatic priming. Nature. https://doi.org/10.1038/s41586-019-1620-6

Bengsch, B., Johnson, A.L., Kurachi, M., Odorizzi, P.M., Pauken, K.E., Attanasio, J., Stelekati, E., McLane, L.M., Paley, M.A., Delgoffe, G.M., Wherry, E.J., 2016. Bioenergetic Insufficiencies Due to Metabolic Alterations Regulated by the Inhibitory Receptor PD-1 Are an Early Driver of CD8+ T Cell Exhaustion. Immunity 45, 358–373. https://doi.org/10.1016/j.immuni.2016.07.008

Bengsch, B., Martin, B., Thimme, R., 2014. Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation. J. Hepatol. https://doi.org/10.1016/j.jhep.2014.07.005

Berges, C., Haberstock, H., Fuchs, D., Sadeghi, M., Opelz, G., Daniel, V., Naujokat, C., 2009. Proteasome inhibition activates the mitochondrial pathway of apoptosis in human CD4+ T cells. J. Cell. Biochem. 108, 935–46. https://doi.org/10.1002/jcb.22325

Bertoletti, A., Ferrari, C., 2016. Adaptive immunity in HBV infection. J. Hepatol. https://doi.org/10.1016/j.jhep.2016.01.026

Bertoletti, A., Tan, A.T., Koh, S., 2017. T-cell therapy for chronic viral hepatitis. Cytotherapy 19, 1317–1324. https://doi.org/10.1016/j.jcyt.2017.07.011

Blackburn, S.D., Shin, H., Freeman, G.J., Wherry, E.J., 2008. Selective expansion of a subset of exhausted CD8 T cells by αPD-L1 blockade. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.0801497105

Blackburn, S.D., Shin, H., Haining, W.N., Zou, T., Workman, C.J., Polley, A., Betts, M.R., Freeman, G.J., Vignali, D.A.A., Wherry, E.J., 2009. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat. Immunol. 10, 29–37.

https://doi.org/10.1038/ni.1679

Blagih, J., Coulombe, F., Vincent, E.E., Dupuy, F., Galicia-Vázquez, G., Yurchenko, E., Raissi, T.C., vanderWindt, G.J.W., Viollet, B., Pearce, E.L., Pelletier, J., Piccirillo, C.A., Krawczyk, C.M., Divangahi, M., Jones, R.G., 2015. The Energy Sensor AMPK Regulates T Cell Metabolic Adaptation and Effector Responses InVivo. Immunity. https://doi.org/10.1016/j.immuni.2014.12.030

Blagih, J., Krawczyk, C.M., Jones, R.G., 2012. LKB1 and AMPK: central regulators of lymphocyte metabolism and function. Immunol. Rev. 249, 59–71. https://doi.org/10.1111/j.1600-065X.2012.01157.x

Bohne, F., Chmielewski, M., Ebert, G., Wiegmann, K., Kürschner, T., Schulze, A., Urban, S., Krönke, M., Abken, H., Protzer, U., 2008. T cells redirected against hepatitis B virus surface proteins eliminate infected hepatocytes. Gastroenterology 134, 239–47. https://doi.org/10.1053/j.gastro.2007.11.002

Boni, C., Barili, V., Acerbi, G., Rossi, M., Vecchi, A., Laccabue, D., Penna, A., Missale, G., Ferrari, C., Fisicaro, P., 2019a. HBV Immune-Therapy: From Molecular Mechanisms to Clinical Applications. Int. J. Mol. Sci. 20, 2754. https://doi.org/10.3390/ijms20112754

Boni, C., Barili, V., Acerbi, G., Rossi, M., Vecchi, A., Laccabue, D., Penna, A., Missale, G., Ferrari, C., Fisicaro, P., 2019b. HBV Immune-Therapy: From Molecular Mechanisms to Clinical Applications. Int. J. Mol. Sci. 20, 2754. https://doi.org/10.3390/ijms20112754

Boni, C., Fisicaro, P., Valdatta, C., Amadei, B., Di Vincenzo, P., Giuberti, T., Laccabue, D., Zerbini, A., Cavalli, A., Missale, G., Bertoletti, A., Ferrari, C., 2007. Characterization of Hepatitis B Virus (HBV)-Specific T-Cell Dysfunction in Chronic HBV Infection. J. Virol. 81, 4215–4225. https://doi.org/10.1128/JVI.02844-06

Boni, C., Janssen, H.L.A., Rossi, M., Yoon, S.K., Vecchi, A., Barili, V., Yoshida, E.M., Trinh, H., Rodell, T.C., Laccabue, D., Alfieri, A., Brillo, F., Fisicaro, P., Acerbi, G., Pedrazzi, G., Andreone, P., Cursaro, C., Margotti, M., Santoro, R., Piazzolla, V., Brunetto, M.R., Coco, B., Cavallone, D., Zhao, Y., Joshi, A., Woo, J., Lau, A.H., Gaggar, A., Subramanian, G.M., Massetto, B., Fung, S., Ahn, S.H., Ma, X., Mangia, A., Ferrari, C., 2019c. Combined GS-4774 and Tenofovir Therapy Can Improve HBV-Specific T-Cell Responses in Patients With Chronic Hepatitis. Gastroenterology 157, 227–241.e7. https://doi.org/10.1053/j.gastro.2019.03.044

Boni, C., Lampertico, P., Talamona, L., Giuberti, T., Invernizzi, F., Barili, V., Fisicaro, P., Rossi, M.,

Cavallo, M.C., Vecchi, A., Pedrazzi, G., Alfieri, A., Colombo, M., Missale, G., Ferrari, C., 2015. Natural killer cell phenotype modulation and natural killer/T-cell interplay in nucleos(t)ide analogue-treated hepatitis e antigen-negative patients with chronic hepatitis B. Hepatology 62, 1697–1709. https://doi.org/10.1002/hep.28155

Bordon, Y., 2019. TOX for tired T cells. Nat. Rev. Immunol. https://doi.org/10.1038/s41577-019-0193-9

Bronietzki, A.W., Schuster, M., Schmitz, I., 2015. Autophagy in T-cell development, activation and differentiation. Immunol. Cell Biol. 93, 25–34. https://doi.org/10.1038/icb.2014.81

Bruckbauer, A., Zemel, M.B., 2014. Synergistic effects of polyphenols and methylxanthines with Leucine on AMPK/Sirtuin-mediated metabolism in muscle cells and adipocytes. PLoS One 9, e89166. https://doi.org/10.1371/journal.pone.0089166

Buck, M.D., O'Sullivan, D., Pearce, E.L., 2015. T cell metabolism drives immunity. J. Exp. Med. https://doi.org/10.1084/jem.20151159

Buck, M.D., Sowell, R.T., Kaech, S.M., Pearce, E.L., 2017. Metabolic Instruction of Immunity. Cell 169, 570–586. https://doi.org/10.1016/j.cell.2017.04.004

Buck, M.D.D., O'Sullivan, D., Klein Geltink, R.I.I., Curtis, J.D.D., Chang, C.H., Sanin, D.E.E., Qiu, J., Kretz, O., Braas, D., van der Windt, G.J.J.W., Chen, Q., Huang, S.C.C., O'Neill, C.M.M., Edelson, B.T.T., Pearce, E.J.J., Sesaki, H., Huber, T.B.B., Rambold, A.S.S., Pearce, E.L.L., 2016. Mitochondrial Dynamics Controls T Cell Fate through Metabolic Programming. Cell 166, 63–76. https://doi.org/10.1016/j.cell.2016.05.035

Caldwell, C.C., Kojima, H., Lukashev, D., Armstrong, J., Farber, M., Apasov, S.G., Sitkovsky, M. V., 2001. Differential Effects of Physiologically Relevant Hypoxic Conditions on T Lymphocyte Development and Effector Functions. J. Immunol. https://doi.org/10.4049/jimmunol.167.11.6140

Carr, E.L., Kelman, A., Wu, G.S., Gopaul, R., Senkevitch, E., Aghvanyan, A., Turay, A.M., Frauwirth, K.A., 2010. Glutamine Uptake and Metabolism Are Coordinately Regulated by ERK/MAPK during T Lymphocyte Activation. J. Immunol. https://doi.org/10.4049/jimmunol.0903586

Cavenaugh, J.S., Awi, D., Mendy, M., Hill, A.V.S., Whittle, H., McConkey, S.J., 2011. Partially randomized, non-blinded trial of DNA and MVA therapeutic vaccines based on hepatitis B virus surface protein for chronic HBV infection. PLoS One. https://doi.org/10.1371/journal.pone.0014626

Chang, C.-H., Curtis, J.D., Maggi, L.B., Faubert, B., Villarino, A. V., O'Sullivan, D., Huang, S.C.-C., van der Windt, G.J.W., Blagih, J., Qiu, J., Weber, J.D., Pearce, E.J., Jones, R.G., Pearce, E.L., 2013. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 153, 1239–51.

https://doi.org/10.1016/j.cell.2013.05.016

Chang, C.H., Pearce, E.L., 2016. Emerging concepts of T cell metabolism as a target of immunotherapy. Nat. Immunol. https://doi.org/10.1038/ni.3415

Chang, C.H., Qiu, J., O'Sullivan, D., Buck, M.D., Noguchi, T., Curtis, J.D., Chen, Q., Gindin, M., Gubin, M.M., Van Der Windt, G.J.W., Tonc, E., Schreiber, R.D., Pearce, E.J., Pearce, E.L., 2015. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. Cell. https://doi.org/10.1016/j.cell.2015.08.016

Chapman, N.M., Boothby, M.R., Chi, H., 2020. Metabolic coordination of T cell quiescence and activation. Nat. Rev. Immunol. 20, 55–70. https://doi.org/10.1038/s41577-019-0203-y

Chemnitz, J.M., Parry, R. V., Nichols, K.E., June, C.H., Riley, J.L., 2004. SHP-1 and SHP-2 Associate with Immunoreceptor Tyrosine-Based Switch Motif of Programmed Death 1 upon Primary Human T Cell Stimulation, but Only Receptor Ligation Prevents T Cell Activation. J. Immunol. https://doi.org/10.4049/jimmunol.173.2.945

Chen, C., Lou, T., 2017. Hypoxia inducible factors in hepatocellular carcinoma. Oncotarget 8, 46691–46703. https://doi.org/10.18632/oncotarget.17358

Cheng, Y., Zhu, Y.O., Becht, E., Aw, P., Chen, J., Poidinger, M., de Sessions, P.F., Hibberd, M.L., Bertoletti, A., Lim, S.G., Newell, E.W., 2019. Multifactorial heterogeneity of virus-specific T cells and association with the progression of human chronic hepatitis B infection. Sci. Immunol. 4, eaau6905. https://doi.org/10.1126/sciimmunol.aau6905

Chisolm, D.A., Weinmann, A.S., 2018. Connections Between Metabolism and Epigenetics in Programming Cellular Differentiation. Annu. Rev. Immunol. 36, 221–246. https://doi.org/10.1146/annurev-immunol-042617-053127

Chiu, D.K.-C., Tse, A.P.-W., Xu, I.M.-J., Di Cui, J., Lai, R.K.-H., Li, L.L., Koh, H.-Y., Tsang, F.H.-C., Wei, L.L., Wong, C.-M., Ng, I.O.-L., Wong, C.C.-L., 2017. Hypoxia inducible factor HIF-1 promotes myeloid-derived suppressor cells accumulation through ENTPD2/CD39L1 in hepatocellular carcinoma. Nat. Commun. 8, 517. https://doi.org/10.1038/s41467-017-00530-7

Chouaib, S., Noman, M.Z., Kosmatopoulos, K., Curran, M.A., 2017. Hypoxic stress: obstacles and opportunities for innovative immunotherapy of cancer. Oncogene 36, 439–445. https://doi.org/10.1038/onc.2016.225

Choudhry, H., Harris, A.L., 2018. Advances in Hypoxia-Inducible Factor Biology. Cell Metab. 27, 281–298. https://doi.org/10.1016/j.cmet.2017.10.005

Clambey, E.T., McNamee, E.N., Westrich, J.A., Glover, L.E., Campbell, E.L., Jedlicka, P., De Zoeten, E.F., Cambier, J.C., Stenmark, K.R., Colgan, S.P., Eltzschig, H.K., 2012. Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1202366109

Cretenet, G., Clerc, I., Matias, M., Loisel, S., Craveiro, M., Oburoglu, L., Kinet, S., Mongellaz, C., Dardalhon, V., Taylor, N., 2016. Cell surface Glut1 levels distinguish human CD4 and CD8 T lymphocyte subsets with distinct effector functions. Sci. Rep. 6, 24129. https://doi.org/10.1038/srep24129

D'Amico, D., Sorrentino, V., Auwerx, J., 2017. Cytosolic Proteostasis Networks of the Mitochondrial Stress Response. Trends Biochem. Sci. https://doi.org/10.1016/j.tibs.2017.05.002

Dang, E. V., Barbi, J., Yang, H.-Y., Jinasena, D., Yu, H., Zheng, Y., Bordman, Z., Fu, J., Kim, Y., Yen, H.-R., Luo, W., Zeller, K., Shimoda, L., Topalian, S.L., Semenza, G.L., Dang, C. V., Pardoll, D.M., Pan, F., 2011. Control of TH17/Treg Balance by Hypoxia-Inducible Factor 1. Cell 146, 772–784. https://doi.org/10.1016/j.cell.2011.07.033

de silly, R.V., Ducimetière, L., Yacoub maroun, C., Dietrich, P.Y., Derouazi, M., Walker, P.R., 2015. Phenotypic switch of CD8+ T cells reactivated under hypoxia toward IL-10 secreting, poorly proliferative effector cells. Eur. J. Immunol. https://doi.org/10.1002/eji.201445284

Dembek, C., Protzer, U., Roggendorf, M., 2018. Overcoming immune tolerance in chronic hepatitis B by therapeutic vaccination. Curr. Opin. Virol. https://doi.org/10.1016/j.coviro.2018.04.003

Dhouafli, Z., Cuanalo-Contreras, K., Hayouni, E.A., Mays, C.E., Soto, C., Moreno-Gonzalez, I., 2018. Inhibition of protein misfolding and aggregation by natural phenolic compounds. Cell. Mol. Life Sci. 75, 3521–3538. https://doi.org/10.1007/s00018-018-2872-2

Dimeloe, S., Mehling, M., Frick, C., Loeliger, J., Bantug, G.R., Sauder, U., Fischer, M., Belle, R., Develioglu, L., Tay, S., Langenkamp, A., Hess, C., 2016. The Immune-Metabolic Basis of Effector Memory CD4 + T Cell Function under Hypoxic Conditions. J. Immunol. 196, 106–114. https://doi.org/10.4049/jimmunol.1501766

Doedens, A.L., Phan, A.T., Stradner, M.H., Fujimoto, J.K., Nguyen, J. V., Yang, E., Johnson, R.S., Goldrath, A.W., 2013. Hypoxia-inducible factors enhance the effector responses of CD8 + T cells to persistent antigen. Nat. Immunol. https://doi.org/10.1038/ni.2714

Doering, T.A., Crawford, A., Angelosanto, J.M., Paley, M.A., Ziegler, C.G., Wherry, E.J., 2012. Network

Analysis Reveals Centrally Connected Genes and Pathways Involved in CD8+ T Cell Exhaustion versus Memory. Immunity 37, 1130–1144. https://doi.org/10.1016/j.immuni.2012.08.021

Dunn, J., Rao, S., 2017. Epigenetics and immunotherapy: The current state of play. Mol. Immunol. https://doi.org/10.1016/j.molimm.2017.04.012

Egan, D.F., Shackelford, D.B., Mihaylova, M.M., Gelino, S., Kohnz, R.A., Mair, W., Vasquez, D.S., Joshi, A., Gwinn, D.M., Taylor, R., Asara, J.M., Fitzpatrick, J., Dillin, A., Viollet, B., Kundu, M., Hansen, M., Shaw, R.J., 2011. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science (80-.). https://doi.org/10.1126/science.1196371

Esensten, J.H., Helou, Y.A., Chopra, G., Weiss, A., Bluestone, J.A., 2016. CD28 Costimulation: From Mechanism to Therapy. Immunity 44, 973–988. https://doi.org/10.1016/j.immuni.2016.04.020

Evans, A., Riva, A., Cooksley, H., Phillips, S., Puranik, S., Nathwani, A., Brett, S., Chokshi, S., Naoumov, N. V., 2008. Programmed death 1 expression during antiviral treatment of chronic hepatitis B: Impact of hepatitis B e-antigen seroconversion. Hepatology 48, 759–769. https://doi.org/10.1002/hep.22419

Fallarino, F., Grohmann, U., Vacca, C., Bianchi, R., Orabona, C., Spreca, A., Fioretti, M.C., Puccetti, P., 2002. T cell apoptosis by tryptophan catabolism. Cell Death Differ. 9, 1069–1077. https://doi.org/10.1038/sj.cdd.4401073

Fallarino, F., Grohmann, U., You, S., McGrath, B.C., Cavener, D.R., Vacca, C., Orabona, C., Bianchi, R., Belladonna, M.L., Volpi, C., Santamaria, P., Fioretti, M.C., Puccetti, P., 2006. The Combined Effects of Tryptophan Starvation and Tryptophan Catabolites Down-Regulate T Cell Receptor ζ-Chain and Induce a Regulatory Phenotype in Naive T Cells. J. Immunol. 176, 6752–6761. https://doi.org/10.4049/jimmunol.176.11.6752

Fanning, G.C., Zoulim, F., Hou, J., Bertoletti, A., 2019. Therapeutic strategies for hepatitis B virus infection: towards a cure. Nat. Rev. Drug Discov. https://doi.org/10.1038/s41573-019-0037-0

Feng, C., Cao, L.-J., Song, H.-F., Xu, P., Chen, H., Xu, J.-C., Zhu, X.-Y., Zhang, X.-G., Wang, X.-F., 2015. Expression of PD-L1 on CD4+CD25+Foxp3+ Regulatory T Cells of Patients with Chronic HBV Infection and Its Correlation with Clinical Parameters. Viral Immunol. 28, 418–24. https://doi.org/10.1089/vim.2015.0062

Fernandez-Mosquera, L., Yambire, K.F., Couto, R., Pereyra, L., Pabis, K., Ponsford, A.H., Diogo, C. V., Stagi, M., Milosevic, I., Raimundo, N., 2019. Mitochondrial respiratory chain deficiency inhibits lysosomal hydrolysis. Autophagy. https://doi.org/10.1080/15548627.2019.1586256

Ferrari, C., Boni, C., Rossi, M., Vecchi, A., Barili, V., Laccabue, D., Fisicaro, P., Missale, G., 2017. T cell regulation in HBV-related chronic liver disease. J. Hepatol. 66. https://doi.org/10.1016/j.jhep.2016.10.002

Festag, M.M., Festag, J., Fräßle, S.P., Asen, T., Sacherl, J., Schreiber, S., Mück-Häusl, M.A., Busch, D.H., Wisskirchen, K., Protzer, U., 2019. Evaluation of a Fully Human, Hepatitis B Virus-Specific Chimeric Antigen Receptor in an Immunocompetent Mouse Model. Mol. Ther. 27, 947–959. https://doi.org/10.1016/j.ymthe.2019.02.001

Finlay, D., Cantrell, D.A., 2011. Metabolism, migration and memory in cytotoxic T cells. Nat. Rev. Immunol. 11, 109–117. https://doi.org/10.1038/nri2888

Finlay, D.K., Rosenzweig, E., Sinclair, L. V., Feijoo-Carnero, C., Hukelmann, J.L., Rolf, J., Panteleyev, A.A., Okkenhaug, K., Cantrell, D.A., 2012. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. J. Exp. Med. 209, 2441–2453. https://doi.org/10.1084/jem.20112607

Fisicaro, P., Barili, V., Acerbi, G., Montanini, B., Laccabue, D., Guerrieri, F., Massari, M., Vandelli, C., Salerno, D., Grossi, G., Alfieri, A., Lampertico, P., Giuberti, T., Missale, G., Levrero, M., Ottonello, S., Ferrari, C., 2017. Proteasome dysfunction as a reversible defect underlying virus-specific CD8 cell exhaustion in chronic hepatitis B. J. Hepatol. 66, S30. https://doi.org/10.1016/S0168-8278(17)30324-0

Fisicaro, P., Barili, V., Montanini, B., Acerbi, G., Ferracin, M., Guerrieri, F., Salerno, D., Boni, C., Massari, M., Cavallo, M.C., Grossi, G., Giuberti, T., Lampertico, P., Missale, G., Levrero, M., Ottonello, S., Ferrari, C., 2017a. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. Nat. Med. 23, 327–336. https://doi.org/10.1038/nm.4275

Fisicaro, P., Barili, V., Montanini, B., Acerbi, G., Ferracin, M., Guerrieri, F., Salerno, D., Boni, C., Massari, M., Cavallo, M.C., Grossi, G., Giuberti, T., Lampertico, P., Missale, G., Levrero, M., Ottonello, S., Ferrari, C., 2017b. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. Nat. Med. 23, 327–336. https://doi.org/10.1038/nm.4275

Fisicaro, P., Barili, V., Rossi, M., Montali, I., Vecchi, A., Acerbi, G., Laccabue, D., Zecca, A., Penna, A., Missale, G., Ferrari, C., Boni, C., 2020. Pathogenetic Mechanisms of T Cell Dysfunction in Chronic HBV Infection and Related Therapeutic Approaches. Front. Immunol. 11, 849. https://doi.org/10.3389/fimmu.2020.00849

Fisicaro, P., Boni, C., Barili, V., Laccabue, D., Ferrari, C., 2018. Strategies to overcome HBV-specific T cell exhaustion: checkpoint inhibitors and metabolic re-programming. Curr. Opin. Virol. https://doi.org/10.1016/j.coviro.2018.01.003

Fisicaro, P., Valdatta, C., Massari, M., Loggi, E., Biasini, E., Sacchelli, L., Cavallo, M.C., Silini, E.M., Andreone, P., Missale, G., Ferrari, C., 2010. Antiviral Intrahepatic T-Cell Responses Can Be Restored by Blocking Programmed Death-1 Pathway in Chronic Hepatitis B. Gastroenterology 138, 682–693.e4. https://doi.org/10.1053/j.gastro.2009.09.052

Fisicaro, P., Valdatta, C., Massari, M., Loggi, E., Ravanetti, L., Urbani, S., Giuberti, T., Cavalli, A., Vandelli, C., Andreone, P., Missale, G., Ferrari, C., 2012. Combined Blockade of Programmed Death-1 and Activation of CD137 Increase Responses of Human Liver T Cells Against HBV, But Not HCV. Gastroenterology 143, 1576–1585.e4. https://doi.org/10.1053/j.gastro.2012.08.041

Fontaine, H., Kahi, S., Chazallon, C., Bourgine, M., Varaut, A., Buffet, C., Godon, O., Meritet, J.F., Saïdi, Y., Michel, M.L., Scott-Algara, D., Aboulker, J.P., Pol, S., 2015. Anti-HBV DNA vaccination does not prevent relapse after discontinuation of analogues in the treatment of chronic hepatitis B: a randomised trial—ANRS HB02 VAC-ADN. Gut 64, 139–147. https://doi.org/10.1136/gutjnl-2013-305707

Frauwirth, K.A., Riley, J.L., Harris, M.H., Parry, R. V., Rathmell, J.C., Plas, D.R., Elstrom, R.L., June, C.H., Thompson, C.B., 2002. The CD28 Signaling Pathway Regulates Glucose Metabolism. Immunity 16, 769–777. https://doi.org/10.1016/S1074-7613(02)00323-0

Gabrilovich, D.I., Nagaraj, S., 2009. Myeloid-derived suppressor cells as regulators of the immune system. Nat. Rev. Immunol. 9, 162–74. https://doi.org/10.1038/nri2506

Ganeshan, K., Chawla, A., 2014. Metabolic Regulation of Immune Responses. Annu. Rev. Immunol. 32, 609–634. https://doi.org/10.1146/annurev-immunol-032713-120236

Gehring, A.J., Protzer, U., 2019. Targeting Innate and Adaptive Immune Responses to Cure Chronic HBV Infection. Gastroenterology. https://doi.org/10.1053/j.gastro.2018.10.032

Gehring, A.J., Xue, S.-A., Ho, Z.Z., Teoh, D., Ruedl, C., Chia, A., Koh, S., Lim, S.G., Maini, M.K., Stauss, H., Bertoletti, A., 2011. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. J. Hepatol. 55, 103–110. https://doi.org/10.1016/j.jhep.2010.10.025

Ghoneim, H.E., Fan, Y., Moustaki, A., Abdelsamed, H.A., Dash, P., Dogra, P., Carter, R., Awad, W., Neale, G., Thomas, P.G., Youngblood, B., 2017. De Novo Epigenetic Programs Inhibit PD-1

Blockade-Mediated T Cell Rejuvenation. Cell 170, 142–157.e19. https://doi.org/10.1016/j.cell.2017.06.007

Gill, U.S., Pallett, L.J., Thomas, N., Burton, A.R., Patel, A.A., Yona, S., Kennedy, P.T.F., Maini, M.K., 2019. Fine needle aspirates comprehensively sample intrahepatic immunity. Gut. https://doi.org/10.1136/gutjnl-2018-317071

Godon, O., Fontaine, H., Kahi, S., Meritet, J., Scott-Algara, D., Pol, S., Michel, M., Bourgine, M., 2014. Immunological and Antiviral Responses After Therapeutic DNA Immunization in Chronic Hepatitis B Patients Efficiently Treated by Analogues. Mol. Ther. 22, 675–684. https://doi.org/10.1038/mt.2013.274

Goswami, S., Apostolou, I., Zhang, J., Skepner, J., Anandhan, S., Zhang, X., Xiong, L., Trojer, P., Aparicio, A., Subudhi, S.K., Allison, J.P., Zhao, H., Sharma, P., 2018. Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy. J. Clin. Invest. https://doi.org/10.1172/JCI99760

Gropper, Y., Feferman, T., Shalit, T., Salame, T.-M., Porat, Z., Shakhar, G., 2017. Culturing CTLs under Hypoxic Conditions Enhances Their Cytolysis and Improves Their Anti-tumor Function. Cell Rep. 20, 2547–2555. https://doi.org/10.1016/j.celrep.2017.08.071

Gubser, P.M., Bantug, G.R., Razik, L., Fischer, M., Dimeloe, S., Hoenger, G., Durovic, B., Jauch, A., Hess, C., 2013. Rapid effector function of memory CD8+T cells requires an immediate-early glycolytic switch. Nat. Immunol. 14, 1064–1072. https://doi.org/10.1038/ni.2687

Guidotti, L.G., Chisari, F. V., 2006. IMMUNOBIOLOGY AND PATHOGENESIS OF VIRAL HEPATITIS. Annu. Rev. Pathol. Mech. Dis. 1, 23–61. https://doi.org/10.1146/annurev.pathol.1.110304.100230

Gumeni, S., Trougakos, I.P., 2016. Cross Talk of Proteostasis and Mitostasis in Cellular Homeodynamics, Ageing, and Disease. Oxid. Med. Cell. Longev. 2016, 4587691. https://doi.org/10.1155/2016/4587691

Hardie, D.G., 2011a. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev. 25, 1895–908. https://doi.org/10.1101/gad.17420111

Hardie, D.G., 2011b. AMPK and autophagy get connected. EMBO J. https://doi.org/10.1038/emboj.2011.12

Heymann, F., Tacke, F., 2016. Immunology in the liver-from homeostasis to disease. Nat. Rev. Gastroenterol. Hepatol. https://doi.org/10.1038/nrgastro.2015.200

Holz, L.E., Benseler, V., Bowen, D.G., Bouillet, P., Strasser, A., O'Reilly, L., D'Avigdor, W.M.H., Bishop, A.G., McCaughan, G.W., Bertolino, P., 2008. Intrahepatic Murine CD8 T-Cell Activation Associates With a Distinct Phenotype Leading to Bim-Dependent Death. Gastroenterology 135, 989–997. https://doi.org/10.1053/j.gastro.2008.05.078

Honda, T., Egen, J.G., Lämmermann, T., Kastenmüller, W., Torabi-Parizi, P., Germain, R.N., 2014. Tuning of Antigen Sensitivity by T Cell Receptor-Dependent Negative Feedback Controls T Cell Effector Function in Inflamed Tissues. Immunity. https://doi.org/10.1016/j.immuni.2013.11.017

Hoogeveen, R.C., Boonstra, A., 2020. Checkpoint Inhibitors and Therapeutic Vaccines for the Treatment of Chronic HBV Infection. Front. Immunol. https://doi.org/10.3389/fimmu.2020.00401

Hoogeveen, R.C., Robidoux, M.P., Schwarz, T., Heydmann, L., Cheney, J.A., Kvistad, D., Aneja, J., Melgaço, J.G., Fernandes, C.A., Chung, R.T., Boonstra, A., Kim, A.Y., Baumert, T.F., Timm, J., Lewis-Ximenez, L.L., Tonnerre, P., Lauer, G.M., 2018. Phenotype and function of HBV-specific T cells is determined by the targeted epitope in addition to the stage of infection. Gut. https://doi.org/10.1136/gutjnl-2018-316644

Horst, A.K., Neumann, K., Diehl, L., Tiegs, G., 2016. Modulation of liver tolerance by conventional and nonconventional antigen-presenting cells and regulatory immune cells. Cell. Mol. Immunol. 13, 277–292. https://doi.org/10.1038/cmi.2015.112

Huang, W.-C., Easom, N.J., Tang, X.-Z., Gill, U.S., Singh, H., Robertson, F., Chang, C., Trowsdale, J., Davidson, B.R., Rosenberg, W.M., Fusai, G., Toubert, A., Kennedy, P.T., Peppa, D., Maini, M.K., 2017. T Cells Infiltrating Diseased Liver Express Ligands for the NKG2D Stress Surveillance System. J. Immunol. https://doi.org/10.4049/jimmunol.1601313

Huang, Z.-Y., Xu, P., Li, J.-H., Zeng, C.-H., Song, H.-F., Chen, H., Zhu, Y.-B., Song, Y.-Y., Lu, H.-L., Shen, C.-P., Zhang, X.-G., Wu, M.-Y., Wang, X.-F., 2017. Clinical Significance of Dynamics of Programmed Death Ligand-1 Expression on Circulating CD14 + Monocytes and CD19 + B Cells with the Progression of Hepatitis B Virus Infection. Viral Immunol. 30, 224–231. https://doi.org/10.1089/vim.2016.0122

Huemer, F., Leisch, M., Geisberger, R., Melchardt, T., Rinnerthaler, G., Zaborsky, N., Greil, R., 2020. Combination strategies for immune-checkpoint blockade and response prediction by artificial intelligence. Int. J. Mol. Sci. https://doi.org/10.3390/ijms21082856

Hui, E., Cheung, J., Zhu, J., Su, X., Taylor, M.J., Wallweber, H.A., Sasmal, D.K., Huang, J., Kim, J.M., Mellman, I., Vale, R.D., 2017. T cell costimulatory receptor CD28 is a primary target for PD-1-

mediated inhibition. Science (80-.). https://doi.org/10.1126/science.aaf1292

Jacobi, F.J., Wild, K., Smits, M., Zoldan, K., Csernalabics, B., Flecken, T., Lang, J., Ehrenmann, P., Emmerich, F., Hofmann, M., Thimme, R., Neumann-Haefelin, C., Boettler, T., 2019. OX40 stimulation and PD-L1 blockade synergistically augment HBV-specific CD4 T cells in patients with HBeAg-negative infection. J. Hepatol. 70, 1103–1113. https://doi.org/10.1016/j.jhep.2019.02.016

Jacobs, S.R., Herman, C.E., Maciver, N.J., Wofford, J.A., Wieman, H.L., Hammen, J.J., Rathmell, J.C., 2008. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. J. Immunol. 180, 4476–86. https://doi.org/10.4049/jimmunol.180.7.4476

Jacobs, S.R., Michalek, R.D., Rathmell, J.C., 2010. IL-7 Is Essential for Homeostatic Control of T Cell Metabolism In Vivo. J. Immunol. 184, 3461–3469. https://doi.org/10.4049/jimmunol.0902593

Jo, J., Tan, A.T., Ussher, J.E., Sandalova, E., Tang, X.-Z., Tan-Garcia, A., To, N., Hong, M., Chia, A., Gill, U.S., Kennedy, P.T., Tan, K.C., Lee, K.H., De Libero, G., Gehring, A.J., Willberg, C.B., Klenerman, P., Bertoletti, A., 2014. Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. PLoS Pathog. 10, e1004210. https://doi.org/10.1371/journal.ppat.1004210

Jones, R.G., Pearce, E.J., 2017. MenTORing Immunity: mTOR Signaling in the Development and Function of Tissue-Resident Immune Cells. Immunity. https://doi.org/10.1016/j.immuni.2017.04.028

Ju, C., Colgan, S.P., Eltzschig, H.K., 2016. Hypoxia-inducible factors as molecular targets for liver diseases. J. Mol. Med. 94, 613–627. https://doi.org/10.1007/s00109-016-1408-1

Kaech, S.M., Cui, W., 2012. Transcriptional control of effector and memory CD8+ T cell differentiation. Nat. Rev. Immunol. 12, 749–761. https://doi.org/10.1038/nri3307

Kah, J., Koh, S., Volz, T., Ceccarello, E., Allweiss, L., Lütgehetmann, M., Bertoletti, A., Dandri, M., 2017. Lymphocytes transiently expressing virus-specific T cell receptors reduce hepatitis B virus infection. J. Clin. Invest. 127, 3177–3188. https://doi.org/10.1172/JCI93024

Kalia, V., Sarkar, S., Subramaniam, S., Haining, W.N., Smith, K.A., Ahmed, R., 2010. Prolonged Interleukin-2Rα Expression on Virus-Specific CD8+ T Cells Favors Terminal-Effector Differentiation In Vivo. Immunity 32, 91–103. https://doi.org/10.1016/j.immuni.2009.11.010

Keir, M.E., Butte, M.J., Freeman, G.J., Sharpe, A.H., 2008. PD-1 and its ligands in tolerance and immunity. Annu. Rev. Immunol. 26, 677–704. https://doi.org/10.1146/annurev.immunol.26.021607.090331

Khan, O., Giles, J.R., McDonald, S., Manne, S., Ngiow, S.F., Patel, K.P., Werner, M.T., Huang, A.C., Alexander, K.A., Wu, J.E., Attanasio, J., Yan, P., George, S.M., Bengsch, B., Staupe, R.P., Donahue, G., Xu, W., Amaravadi, R.K., Xu, X., Karakousis, G.C., Mitchell, T.C., Schuchter, L.M., Kaye, J., Berger, S.L., Wherry, E.J., 2019. TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. Nature 571, 211–218. https://doi.org/10.1038/s41586-019-1325-x

Kim, J., Kundu, M., Viollet, B., Guan, K.L., 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat. Cell Biol. https://doi.org/10.1038/ncb2152

Kim, J.H., Han, J.W., Choi, Y.J., Rha, M.-S., Koh, J.Y., Kim, K.H., Kim, C.G., Lee, Y.J., Kim, A.R., Park, J., Kim, H.K., Min, B.S., Seo, S. Il, Kang, M., Park, H.J., Han, D.H., Kim, S. Il, Kim, M.S., Lee, J.G., Lee, D.H., Kim, W., Park, J.Y., Park, S.-H., Joo, D.J., Shin, E.-C., 2020. Functions of human liver CD69+CD103-CD8+ T cells depend on HIF-2α activity in healthy and pathologic livers. J. Hepatol. 72, 1170–1181. https://doi.org/10.1016/j.jhep.2020.01.010

Kim, J.W., Tchernyshyov, I., Semenza, G.L., Dang, C. V., 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metab. https://doi.org/10.1016/j.cmet.2006.02.002

Klein Geltink, R.I., Kyle, R.L., Pearce, E.L., 2018. Unraveling the Complex Interplay Between T Cell Metabolism and Function. Annu. Rev. Immunol. 36, 461–488. https://doi.org/10.1146/annurev-immunol-042617-053019

Knolle, P.A., Böttcher, J., Huang, L.R., 2015. The role of hepatic immune regulation in systemic immunity to viral infection. Med. Microbiol. Immunol. https://doi.org/10.1007/s00430-014-0371-0

Kocaturk, N.M., Gozuacik, D., 2018. Crosstalk Between Mammalian Autophagy and the Ubiquitin-Proteasome System. Front. Cell Dev. Biol. 6. https://doi.org/10.3389/fcell.2018.00128

Koh, S., Kah, J., Tham, C.Y.L., Yang, N., Ceccarello, E., Chia, A., Chen, M., Khakpoor, A., Pavesi, A., Tan, A.T., Dandri, M., Bertoletti, A., 2018. Nonlytic Lymphocytes Engineered to Express Virus-Specific T-Cell Receptors Limit HBV Infection by Activating APOBEC3. Gastroenterology 155, 180–193.e6. https://doi.org/10.1053/j.gastro.2018.03.027

Korovila, I., Hugo, M., Castro, J.P., Weber, D., Höhn, A., Grune, T., Jung, T., 2017. Proteostasis, oxidative stress and aging. Redox Biol. https://doi.org/10.1016/j.redox.2017.07.008

Kosinska, A.D., Bauer, T., Protzer, U., 2017. Therapeutic vaccination for chronic hepatitis B. Curr. Opin. Virol. https://doi.org/10.1016/j.coviro.2017.03.011

Krebs, K., Böttinger, N., Huang, L.R., Chmielewski, M., Arzberger, S., Gasteiger, G., Jäger, C., Schmitt,

E., Bohne, F., Aichler, M., Uckert, W., Abken, H., Heikenwalder, M., Knolle, P., Protzer, U., 2013. T Cells Expressing a Chimeric Antigen Receptor That Binds Hepatitis B Virus Envelope Proteins Control Virus Replication in Mice. Gastroenterology. https://doi.org/10.1053/j.gastro.2013.04.047

Kruse, R.L., Shum, T., Tashiro, H., Barzi, M., Yi, Z., Whitten-Bauer, C., Legras, X., Bissig-Choisat, B., Garaigorta, U., Gottschalk, S., Bissig, K.-D., 2018. HBsAg-redirected T cells exhibit antiviral activity in HBV-infected human liver chimeric mice. Cytotherapy 20, 697–705. https://doi.org/10.1016/j.jcyt.2018.02.002

Labiano, S., Palazon, A., Melero, I., 2015. Immune response regulation in the tumor microenvironment by hypoxia. Semin. Oncol. https://doi.org/10.1053/j.seminoncol.2015.02.009

Laker, R.C., Drake, J.C., Wilson, R.J., Lira, V.A., Lewellen, B.M., Ryall, K.A., Fisher, C.C., Zhang, M., Saucerman, J.J., Goodyear, L.J., Kundu, M., Yan, Z., 2017. Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. Nat. Commun. 8, 548. https://doi.org/10.1038/s41467-017-00520-9

Lang, P.A., Lang, K.S., Xu, H.C., Grusdat, M., Parish, I.A., Recher, M., Elford, A.R., Dhanji, S., Shaabani, N., Tran, C.W., Dissanayake, D., Rahbar, R., Ghazarian, M., Brüstle, A., Fine, J., Chen, P., Weaver, C.T., Klose, C., Diefenbach, A., Häussinger, D., Carlyle, J.R., Kaech, S.M., Mak, T.W., Ohashi, P.S., 2012. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. Proc. Natl. Acad. Sci. U. S. A. 109, 1210–5. https://doi.org/10.1073/pnas.1118834109

Lee, J., Walsh, M.C., Hoehn, K.L., James, D.E., Wherry, E.J., Choi, Y., 2014. Regulator of Fatty Acid Metabolism, Acetyl Coenzyme A Carboxylase 1, Controls T Cell Immunity. J. Immunol. https://doi.org/10.4049/jimmunol.1302985

Lee, J.W., Ko, J., Ju, C., Eltzschig, H.K., 2019. Hypoxia signaling in human diseases and therapeutic targets. Exp. Mol. Med. 51, 1–13. https://doi.org/10.1038/s12276-019-0235-1

Lee, P., Chandel, N.S., Simon, M.C., 2020. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. Nat. Rev. Mol. Cell Biol. 21, 268–283. https://doi.org/10.1038/s41580-020-0227-y

Levine, B., Deretic, V., 2007. Unveiling the roles of autophagy in innate and adaptive immunity. Nat. Rev. Immunol. 7, 767–77. https://doi.org/10.1038/nri2161

Li, J., Bao, M., Ge, J., Ren, S., Zhou, T., Qi, F., Pu, X., Dou, J., 2017. Research progress of therapeutic vaccines for treating chronic hepatitis B. Hum. Vaccines Immunother.

https://doi.org/10.1080/21645515.2016.1276125

Lobaina, Y., Michel, M.L., 2017. Chronic hepatitis B: Immunological profile and current therapeutic vaccines in clinical trials. Vaccine. https://doi.org/10.1016/j.vaccine.2017.03.049

Lochner, M., Berod, L., Sparwasser, T., 2015. Fatty acid metabolism in the regulation of T cell function. Trends Immunol. 36, 81–91. https://doi.org/10.1016/j.it.2014.12.005

Löffler, A.S., Alers, S., Dieterle, A.M., Keppeler, H., Franz-Wachtel, M., Kundu, M., Campbell, D.G., Wesselborg, S., Alessi, D.R., Stork, B., 2011. Ulk1-mediated phosphorylation of AMPK constitutes a negative regulatory feedback loop. Autophagy 7, 696–706. https://doi.org/10.4161/auto.7.7.15451

Lopes, A.R., Kellam, P., Das, A., Dunn, C., Kwan, A., Turner, J., Peppa, D., Gilson, R.J., Gehring, A., Bertoletti, A., Maini, M.K., 2008. Bim-mediated deletion of antigen-specific CD8+ T cells in patients unable to control HBV infection. J. Clin. Invest. https://doi.org/10.1172/JCI33402

Ma, E.H., Poffenberger, M.C., Wong, A.H.T., Jones, R.G., 2017. The role of AMPK in T cell metabolism and function. Curr. Opin. Immunol. https://doi.org/10.1016/j.coi.2017.04.004

Ma, Y., Galluzzi, L., Zitvogel, L., Kroemer, G., 2013. Autophagy and cellular immune responses. Immunity. https://doi.org/10.1016/j.immuni.2013.07.017

MacIver, N.J., Michalek, R.D., Rathmell, J.C., 2013. Metabolic Regulation of T Lymphocytes. Annu. Rev. Immunol. 31, 259–283. https://doi.org/10.1146/annurev-immunol-032712-095956

Mackman, R.L., Mish, M., Chin, G., Perry, J.K., Appleby, T., Aktoudianakis, V., Metobo, S., Pyun, P., Niu, C., Daffis, S., Yu, H., Zheng, J., Villasenor, A.G., Zablocki, J., Chamberlain, J., Jin, H., Lee, G., Suekawa-Pirrone, K., Santos, R., Delaney, W.E., Fletcher, S.P., 2020. Discovery of GS-9688 (Selgantolimod) as a Potent and Selective Oral Toll-Like Receptor 8 Agonist for the Treatment of Chronic Hepatitis B. J. Med. Chem. 63, 10188–10203. https://doi.org/10.1021/acs.jmedchem.0c00100

Mahtab, M. Al, Akbar, S.M.F., Aguilar, J.C., Guillen, G., Penton, E., Tuero, A., Yoshida, O., Hiasa, Y., Onji, M., 2018. Treatment of chronic hepatitis B naïve patients with a therapeutic vaccine containing HBs and HBc antigens (a randomized, open and treatment controlled phase III clinical trial). PLoS One. https://doi.org/10.1371/journal.pone.0201236

Maier, H., Isogawa, M., Freeman, G.J., Chisari, F. V., 2007. PD-1:PD-L1 interactions contribute to the functional suppression of virus-specific CD8+ T lymphocytes in the liver. J. Immunol. 178, 2714–20. https://doi.org/10.4049/jimmunol.178.5.2714

Maini, M.K., Burton, A.R., 2019. Restoring, releasing or replacing adaptive immunity in chronic hepatitis

B. Nat. Rev. Gastroenterol. Hepatol. https://doi.org/10.1038/s41575-019-0196-9

Maini, M.K., Pallett, L.J., 2018. Defective T-cell immunity in hepatitis B virus infection: why therapeutic vaccination needs a helping hand. Lancet Gastroenterol. Hepatol. https://doi.org/10.1016/S2468-1253(18)30007-4

Majmundar, A.J., Wong, W.J., Simon, M.C., 2010. Hypoxia-Inducible Factors and the Response to Hypoxic Stress. Mol. Cell. https://doi.org/10.1016/j.molcel.2010.09.022

Mak, T.W., Grusdat, M., Duncan, G.S., Dostert, C., Nonnenmacher, Y., Cox, M., Binsfeld, C., Hao, Z., Brüstle, A., Itsumi, M., Jäger, C., Chen, Y., Pinkenburg, O., Camara, B., Ollert, M., Bindslev-Jensen, C., Vasiliou, V., Gorrini, C., Lang, P.A., Lohoff, M., Harris, I.S., Hiller, K., Brenner, D., 2017. Glutathione Primes T Cell Metabolism for Inflammation. Immunity. https://doi.org/10.1016/j.immuni.2017.03.019

Man, K., Kallies, A., 2015. Synchronizing transcriptional control of T cell metabolism and function. Nat. Rev. Immunol. https://doi.org/10.1038/nri3874

Mayer, A., Denanglaire, S., Viollet, B., Leo, O., Andris, F., 2008. AMP-activated protein kinase regulates lymphocyte responses to metabolic stress but is largely dispensable for immune cell development and function. Eur. J. Immunol. 38, 948–956. https://doi.org/10.1002/eji.200738045

McGoverne, I., Dunn, J., Batham, J., Tu, W.J., Chrisp, J., Rao, S., 2020. Epitherapy and immune checkpoint blockade: using epigenetic reinvigoration of exhausted and dysfunctional T cells to reimburse immunotherapy response. BMC Immunol. 21, 22. https://doi.org/10.1186/s12865-020-00353-0

McGowan, D., Herschke, F., Pauwels, F., Stoops, B., Last, S., Pieters, S., Scholliers, A., Thoné, T., Van Schoubroeck, B., De Pooter, D., Mostmans, W., Khamlichi, M.D., Embrechts, W., Dhuyvetter, D., Smyej, I., Arnoult, E., Demin, S., Borghys, H., Fanning, G., Vlach, J., Raboisson, P., 2016. Novel Pyrimidine Toll-like Receptor 7 and 8 Dual Agonists to Treat Hepatitis B Virus. J. Med. Chem. 59, 7936–49. https://doi.org/10.1021/acs.jmedchem.6b00747

McLane, L.M., Abdel-Hakeem, M.S., Wherry, E.J., 2019. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu. Rev. Immunol. https://doi.org/10.1146/annurev-immunol-041015-055318

McNamee, E.N., Korns Johnson, D., Homann, D., Clambey, E.T., 2013. Hypoxia and hypoxia-inducible factors as regulators of T cell development, differentiation, and function. Immunol. Res. 55, 58–70. https://doi.org/10.1007/s12026-012-8349-8

Mehta, M.M., Weinberg, S.E., Chandel, N.S., 2017. Mitochondrial control of immunity: beyond ATP. Nat. Rev. Immunol. 17, 608–620. https://doi.org/10.1038/nri.2017.66

Mellor, A.L., Munn, D.H., 2004. IDO expression by dendritic cells: Tolerance and tryptophan catabolism. Nat. Rev. Immunol. https://doi.org/10.1038/nri1457

Mezrich, J.D., Fechner, J.H., Zhang, X., Johnson, B.P., Burlingham, W.J., Bradfield, C.A., 2010. An Interaction between Kynurenine and the Aryl Hydrocarbon Receptor Can Generate Regulatory T Cells. J. Immunol. https://doi.org/10.4049/jimmunol.0903670

Mills, E.L., Kelly, B., O'Neill, L.A.J., 2017. Mitochondria are the powerhouses of immunity. Nat. Immunol. 18, 488–498. https://doi.org/10.1038/ni.3704

Mohanty, S., Barik, P., Debata, N., Nagarajan, P., Devadas, S., 2020. iCa2+ Flux, ROS and IL-10 Determines Cytotoxic, and Suppressor T Cell Functions in Chronic Human Viral Infections. Front. Immunol. 11, 83. https://doi.org/10.3389/fimmu.2020.00083

Munder, M., Schneider, H., Luckner, C., Giese, T., Langhans, C.D., Fuentes, J.M., Kropf, P., Mueller, I., Kolb, A., Modolell, M., Ho, A.D., 2006. Suppression of T-cell functions by human granulocyte arginase. Blood. https://doi.org/10.1182/blood-2006-11-010389

Münz, C., 2009. Enhancing immunity through autophagy. Annu. Rev. Immunol. 27, 423–49. https://doi.org/10.1146/annurev.immunol.021908.132537

Muschen, M., Warskulat, U., Peters-Regehr, T., Bode, J.G., Kubitz, R., Haussinger, D., 1999. Involvement of CD95 (Apo-1/Fas) ligand expressed by rat Kupffer cells in hepatic immunoregulation. Gastroenterology. https://doi.org/10.1016/S0016-5085(99)70189-7

Nakagawa, Y., Negishi, Y., Shimizu, M., Takahashi, M., Ichikawa, M., Takahashi, H., 2015. Effects of extracellular pH and hypoxia on the function and development of antigen-specific cytotoxic T lymphocytes. Immunol. Lett. https://doi.org/10.1016/j.imlet.2015.07.003

Nath, B., Szabo, G., 2012. Hypoxia and hypoxia inducible factors: Diverse roles in liver diseases. Hepatology. https://doi.org/10.1002/hep.25497

Nebbia, G., Peppa, D., Schurich, A., Khanna, P., Singh, H.D., Cheng, Y., Rosenberg, W., Dusheiko, G., Gilson, R., ChinAleong, J., Kennedy, P., Maini, M.K., 2012. Upregulation of the Tim-3/Galectin-9 Pathway of T Cell Exhaustion in Chronic Hepatitis B Virus Infection. PLoS One. https://doi.org/10.1371/journal.pone.0047648

Noman, M.Z., Desantis, G., Janji, B., Hasmim, M., Karray, S., Dessen, P., Bronte, V., Chouaib, S., 2014. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced: MDSC-

mediated T cell activation. J. Exp. Med. https://doi.org/10.1084/jem.20131916

Noman, M.Z., Hasmim, M., Messai, Y., Terry, S., Kieda, C., Janji, B., Chouaib, S., 2015. Hypoxia: a key player in antitumor immune response. A Review in the Theme: Cellular Responses to Hypoxia. Am. J. Physiol. Physiol. 309, C569–C579. https://doi.org/10.1152/ajpcell.00207.2015

O'Sullivan, D., Pearce, E.L., 2015. Targeting T cell metabolism for therapy. Trends Immunol. https://doi.org/10.1016/j.it.2014.12.004

O'Sullivan, D., vanderWindt, G.W.J., Huang, S.C.C., Curtis, J.D., Chang, C.H., Buck, M.D.L., Qiu, J., Smith, A.M., Lam, W.Y., DiPlato, L.M., Hsu, F.F., Birnbaum, M.J., Pearce, E.J., Pearce, E.L., 2014. Memory $CD8^+$ T Cells Use Cell-Intrinsic Lipolysis to Support the Metabolic Programming Necessary for Development. Immunity 41, 75–88. https://doi.org/10.1016/j.immuni.2014.06.005

Ohta, A., 2016. A Metabolic Immune Checkpoint: Adenosine in Tumor Microenvironment. Front. Immunol. 7. https://doi.org/10.3389/fimmu.2016.00109

Okazaki, T., Chikuma, S., Iwai, Y., Fagarasan, S., Honjo, T., 2013. A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. Nat. Immunol. 14, 1212–1218. https://doi.org/10.1038/ni.2762

Palazon, A., Goldrath, A.W., Nizet, V., Johnson, R.S., 2014. HIF transcription factors, inflammation, and immunity. Immunity 41, 518–28. https://doi.org/10.1016/j.immuni.2014.09.008

Pallett, L.J., Davies, J., Colbeck, E.J., Robertson, F., Hansi, N., Easom, N.J.W., Burton, A.R., Stegmann, K.A., Schurich, A., Swadling, L., Gill, U.S., Male, V., Luong, T., Gander, A., Davidson, B.R., Kennedy, P.T.F., Maini, M.K., 2017. IL-2high tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. J. Exp. Med. 214, 1567–1580. https://doi.org/10.1084/jem.20162115

Pallett, L.J., Gill, U.S., Quaglia, A., Sinclair, L. V., Jover-Cobos, M., Schurich, A., Singh, K.P., Thomas, N., Das, A., Chen, A., Fusai, G., Bertoletti, A., Cantrell, D.A., Kennedy, P.T., Davies, N.A., Haniffa, M., Maini, M.K., 2015. Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells. Nat. Med. 21, 591–600. https://doi.org/10.1038/nm.3856

Pardoll, D.M., 2012. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer. https://doi.org/10.1038/nrc3239

Parry, R. V., Chemnitz, J.M., Frauwirth, K.A., Lanfranco, A.R., Braunstein, I., Kobayashi, S. V., Linsley, P.S., Thompson, C.B., Riley, J.L., 2005. CTLA-4 and PD-1 Receptors Inhibit T-Cell Activation by Distinct Mechanisms. Mol. Cell. Biol. 25, 9543–9553. https://doi.org/10.1128/MCB.25.21.9543-9553.2005

Patsoukis, N., Bardhan, K., Chatterjee, P., Sari, D., Liu, B., Bell, L.N., Karoly, E.D., Freeman, G.J., Petkova, V., Seth, P., Li, L., Boussiotis, V.A., 2015. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat. Commun. 6, 6692. https://doi.org/10.1038/ncomms7692

Pauken, K.E., Sammons, M.A., Odorizzi, P.M., Manne, S., Godec, J., Khan, O., Drake, A.M., Chen, Z., Sen, D.R., Kurachi, M., Barnitz, R.A., Bartman, C., Bengsch, B., Huang, A.C., Schenkel, J.M., Vahedi, G., Haining, W.N., Berger, S.L., Wherry, E.J., 2016. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. Science (80-.). 354, 1160–1165. https://doi.org/10.1126/science.aaf2807

Pearce, E.L., Poffenberger, M.C., Chang, C.-H., Jones, R.G., 2013. Fueling Immunity: Insights into Metabolism and Lymphocyte Function. Science (80-.). 342, 1242454–1242454. https://doi.org/10.1126/science.1242454

Pearce, E.L., Walsh, M.C., Cejas, P.J., Harms, G.M., Shen, H., Wang, L.S., Jones, R.G., Choi, Y., 2009. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460, 103–107. https://doi.org/10.1038/nature08097

Peppa, D., Gil, U.S., Reynolds, G., Easom, N.J.W., Pallett, L.J., Schurich, A., Micco, L., Nebbia, G., Singh, H.D., Adams, D.H., Kennedy, P.T.F., Maini, M.K., 2013. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. J. Exp. Med. https://doi.org/10.1084/jem.20121172

Petrova, V., Annicchiarico-Petruzzelli, M., Melino, G., Amelio, I., 2018. The hypoxic tumour microenvironment. Oncogenesis. https://doi.org/10.1038/s41389-017-0011-9

Phan, A.T., Goldrath, A.W., 2015. Hypoxia-inducible factors regulate T cell metabolism and function. Mol. Immunol. https://doi.org/10.1016/j.molimm.2015.08.004

Pipkin, M.E., Sacks, J.A., Cruz-Guilloty, F., Lichtenheld, M.G., Bevan, M.J., Rao, A., 2010. Interleukin-2 and Inflammation Induce Distinct Transcriptional Programs that Promote the Differentiation of Effector Cytolytic T Cells. Immunity 32, 79–90. https://doi.org/10.1016/j.immuni.2009.11.012

Pollizzi, K.N., Patel, C.H., Sun, I.H., Oh, M.H., Waickman, A.T., Wen, J., Delgoffe, G.M., Powell, J.D., 2015. mTORC1 and mTORC2 selectively regulate CD8+ T cell differentiation. J. Clin. Invest. https://doi.org/10.1172/JCI77746

Pollizzi, K.N., Powell, J.D., 2014. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. Nat. Rev. Immunol. 14, 435–446. https://doi.org/10.1038/nri3701

Pua, H.H., Guo, J., Komatsu, M., He, Y.-W., 2009. Autophagy Is Essential for Mitochondrial Clearance in Mature T Lymphocytes. J. Immunol. 182, 4046–4055. https://doi.org/10.4049/jimmunol.0801143

Puleston, D.J., Zhang, H., Powell, T.J., Lipina, E., Sims, S., Panse, I., Watson, A.S., Cerundolo, V., Townsend, A.R.M., Klenerman, P., Simon, A.K., 2014. Autophagy is a critical regulator of memory CD8+ T cell formation. Elife 3. https://doi.org/10.7554/eLife.03706

Qasim, W., Brunetto, M., Gehring, A.J., Xue, S.-A., Schurich, A., Khakpoor, A., Zhan, H., Ciccorossi, P., Gilmour, K., Cavallone, D., Moriconi, F., Farzhenah, F., Mazzoni, A., Chan, L., Morris, E., Thrasher, A., Maini, M.K., Bonino, F., Stauss, H., Bertoletti, A., 2015. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. J. Hepatol. 62, 486–491. https://doi.org/10.1016/j.jhep.2014.10.001

Ramsay, G., Cantrell, D., 2015. Environmental and metabolic sensors that control T cell biology. Front. Immunol. https://doi.org/10.3389/fimmu.2015.00099

Rao, R.R., Li, Q., Odunsi, K., Shrikant, P.A., 2010. The mTOR Kinase Determines Effector versus Memory CD8+ T Cell Fate by Regulating the Expression of Transcription Factors T-bet and Eomesodermin. Immunity. https://doi.org/10.1016/j.immuni.2009.10.010

Rathmell, J.C., Heiden, M.G.V., Harris, M.H., Frauwirth, K.A., Thompson, C.B., 2000. In the Absence of Extrinsic Signals, Nutrient Utilization by Lymphocytes Is Insufficient to Maintain Either Cell Size or Viability. Mol. Cell 6, 683–692. https://doi.org/10.1016/S1097-2765(00)00066-6

Raud, B., McGuire, P.J., Jones, R.G., Sparwasser, T., Berod, L., 2018. Fatty acid metabolism in CD8 + T cell memory: Challenging current concepts. Immunol. Rev. 283, 213–231. https://doi.org/10.1111/imr.12655

Raziorrouh, B., Schraut, W., Gerlach, T., Nowack, D., Grüner, N.H., Ulsenheimer, A., Zachoval, R., Wächtler, M., Spannagl, M., Haas, J., Diepolder, H.M., Jung, M.-C., 2010. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. Hepatology 52, 1934–1947. https://doi.org/10.1002/hep.23936

Rehermann, B., 2013. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. Nat. Med. 19, 859–868. https://doi.org/10.1038/nm.3251

Rehermann, B., Nascimbeni, M., 2005. Immunology of hepatitis B virus and hepatitis C virus infection. Nat. Rev. Immunol. https://doi.org/10.1038/nri1573

Rehermann, B., Thimme, R., 2019. Insights From Antiviral Therapy Into Immune Responses to Hepatitis B and C Virus Infection. Gastroenterology 156, 369–383.

https://doi.org/10.1053/j.gastro.2018.08.061

Revill, P.A., Chisari, F. V., Block, J.M., Dandri, M., Gehring, A.J., Guo, H., Hu, J., Kramvis, A., Lampertico, P., Janssen, H.L.A., Levrero, M., Li, W., Liang, T.J., Lim, S.G., Lu, F., Penicaud, M.C., Tavis, J.E., Thimme, R., Arbuthnot, P., Boonstra, A., Chang, K.M., Chen, P.J., Glebe, D., Guidotti, L.G., Fellay, J., Ferrari, C., Jansen, L., Lau, D.T.Y., Lok, A.S., Maini, M.K., Mason, W., Matthews, G., Paraskevis, D., Petersen, J., Rehermann, B., Shin, E.C., Thompson, A., van Bömmel, F., Wang, F.S., Watashi, K., Yang, H.C., Yuan, Z., Yuen, M.F., Block, T., Miller, V., Protzer, U., Bréchot, C., Locarnini, S., Peters, M.G., Schinazi, R.F., Zoulim, F., 2019. A global scientific strategy to cure hepatitis B. Lancet Gastroenterol. Hepatol. https://doi.org/10.1016/S2468-1253(19)30119-0

Reznick, R.M., Zong, H., Li, J., Morino, K., Moore, I.K., Yu, H.J., Liu, Z.-X., Dong, J., Mustard, K.J., Hawley, S.A., Befroy, D., Pypaert, M., Hardie, D.G., Young, L.H., Shulman, G.I., 2007. Aging-Associated Reductions in AMP-Activated Protein Kinase Activity and Mitochondrial Biogenesis. Cell Metab. 5, 151–156. https://doi.org/10.1016/j.cmet.2007.01.008

Riley, J.L., 2009. PD-1 signaling in primary T cells. Immunol. Rev. 229, 114–125. https://doi.org/10.1111/j.1600-065X.2009.00767.x

Rolf, J., Zarrouk, M., Finlay, D.K., Foretz, M., Viollet, B., Cantrell, D.A., 2013. AMPKα1: A glucose sensor that controls CD8 T-cell memory. Eur. J. Immunol. https://doi.org/10.1002/eji.201243008

Ron-Harel, N., Santos, D., Ghergurovich, J.M., Sage, P.T., Reddy, A., Lovitch, S.B., Dephoure, N., Satterstrom, F.K., Sheffer, M., Spinelli, J.B., Gygi, S., Rabinowitz, J.D., Sharpe, A.H., Haigis, M.C., 2016. Mitochondrial Biogenesis and Proteome Remodeling Promote One-Carbon Metabolism for T Cell Activation. Cell Metab. 24, 104–117. https://doi.org/10.1016/j.cmet.2016.06.007

Rosmorduc, O., Housset, C., 2010. Hypoxia: A Link between Fibrogenesis, Angiogenesis, and Carcinogenesis in Liver Disease. Semin. Liver Dis. 30, 258–270. https://doi.org/10.1055/s-0030-1255355

Ruan, L., Zhou, C., Jin, E., Kucharavy, A., Zhang, Y., Wen, Z., Florens, L., Li, R., 2017. Cytosolic proteostasis through importing of misfolded proteins into mitochondria. Nature 543, 443–446. https://doi.org/10.1038/nature21695

Schlie, K., Westerback, A., DeVorkin, L., Hughson, L.R., Brandon, J.M., MacPherson, S., Gadawski, I., Townsend, K.N., Poon, V.I., Elrick, M.A., Côté, H.C.F., Abraham, N., Wherry, E.J., Mizushima, N., Lum, J.J., 2015. Survival of Effector CD8 + T Cells during Influenza Infection Is Dependent on Autophagy . J. Immunol. https://doi.org/10.4049/jimmunol.1402571

Schuch, A., Salimi Alizei, E., Heim, K., Wieland, D., Kiraithe, M.M., Kemming, J., Llewellyn-Lacey, S., Sogukpinar, Ö., Ni, Y., Urban, S., Zimmermann, P., Nassal, M., Emmerich, F., Price, D.A., Bengsch, B., Luxenburger, H., Neumann-Haefelin, C., Hofmann, M., Thimme, R., 2019. Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load. Gut 68, 905–915. https://doi.org/10.1136/gutjnl-2018-316641

Schurich, A., Khanna, P., Lopes, A.R., Han, K.J., Peppa, D., Micco, L., Nebbia, G., Kennedy, P.T.F., Geretti, A.-M., Dusheiko, G., Maini, M.K., 2011. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. Hepatology 53, 1494–1503. https://doi.org/10.1002/hep.24249

Schurich, A., Pallett, L.J., Jajbhay, D., Wijngaarden, J., Otano, I., Gill, U.S., Hansi, N., Kennedy, P.T., Nastouli, E., Gilson, R., Frezza, C., Henson, S.M., Maini, M.K., 2016. Distinct Metabolic Requirements of Exhausted and Functional Virus-Specific CD8 T Cells in the Same Host. Cell Rep. 16, 1243–1252. https://doi.org/10.1016/j.celrep.2016.06.078

Schurich, A., Pallett, L.J., Lubowiecki, M., Singh, H.D., Gill, U.S., Kennedy, P.T., Nastouli, E., Tanwar, S., Rosenberg, W., Maini, M.K., 2013. The Third Signal Cytokine IL-12 Rescues the Anti-Viral Function of Exhausted HBV-Specific CD8 T Cells. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1003208

Sekine, T., Perez-Potti, A., Nguyen, S., Gorin, J.-B., Wu, V.H., Gostick, E., Llewellyn-Lacey, S., Hammer, Q., Falck-Jones, S., Vangeti, S., Yu, M., Smed-Sörensen, A., Gaballa, A., Uhlin, M., Sandberg, J.K., Brander, C., Nowak, P., Goepfert, P.A., Price, D.A., Betts, M.R., Buggert, M., 2020. TOX is expressed by exhausted and polyfunctional human effector memory CD8 + T cells. Sci. Immunol. 5, eaba7918. https://doi.org/10.1126/sciimmunol.aba7918

Sen, D.R., Kaminski, J., Barnitz, R.A., Kurachi, M., Gerdemann, U., Yates, K.B., Tsao, H.-W., Godec, J., LaFleur, M.W., Brown, F.D., Tonnerre, P., Chung, R.T., Tully, D.C., Allen, T.M., Frahm, N., Lauer, G.M., Wherry, E.J., Yosef, N., Haining, W.N., 2016. The epigenetic landscape of T cell exhaustion. Science (80-.). 354, 1165–1169. https://doi.org/10.1126/science.aae0491

Sena, L.A., Li, S., Jairaman, A., Prakriya, M., Ezponda, T., Hildeman, D.A., Wang, C.R., Schumacker, P.T., Licht, J.D., Perlman, H., Bryce, P.J., Chandel, N.S., 2013. Mitochondria Are Required for Antigen-Specific T Cell Activation through Reactive Oxygen Species Signaling. Immunity. https://doi.org/10.1016/j.immuni.2012.10.020

Sheppard, K.A., Fitz, L.J., Lee, J.M., Benander, C., George, J.A., Wooters, J., Qiu, Y., Jussif, J.M.,

Carter, L.L., Wood, C.R., Chaudhary, D., 2004. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3ζ signalosome and downstream signaling to PKCθ. FEBS Lett. https://doi.org/10.1016/j.febslet.2004.07.083

Shi, L.Z., Wang, R., Huang, G., Vogel, P., Neale, G., Green, D.R., Chi, H., 2011. HIF1α-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J. Exp. Med. https://doi.org/10.1084/jem.20110278

Shin, E.C., Sung, P.S., Park, S.H., 2016. Immune responses and immunopathology in acute and chronic viral hepatitis. Nat. Rev. Immunol. https://doi.org/10.1038/nri.2016.69

Sinclair, L. V., Rolf, J., Emslie, E., Shi, Y.-B., Taylor, P.M., Cantrell, D.A., 2013. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. Nat. Immunol. 14, 500–508. https://doi.org/10.1038/ni.2556

Siska, P.J., Kim, B., Ji, X., Hoeksema, M.D., Massion, P.P., Beckermann, K.E., Wu, J., Chi, J.-T., Hong, J., Rathmell, J.C., 2016. Fluorescence-based measurement of cystine uptake through xCT shows requirement for ROS detoxification in activated lymphocytes. J. Immunol. Methods 438, 51–58. https://doi.org/10.1016/j.jim.2016.08.013

Sitkovsky, M., Lukashev, D., 2005. Regulation of immune cells by local-tissue oxygen tension: HIF1α and adenosine receptors. Nat. Rev. Immunol. 5, 712–721. https://doi.org/10.1038/nri1685

Sorgdrager, F.J.H., Naudé, P.J.W., Kema, I.P., Nollen, E.A., Deyn, P.P. De, 2019. Tryptophan Metabolism in Inflammaging: From Biomarker to Therapeutic Target. Front. Immunol. 10, 2565. https://doi.org/10.3389/fimmu.2019.02565

Sormendi, S., Wielockx, B., 2016. HIF-pathway proteins: central regulators of tumor immunology. Transl. Cancer Res. 5, S1503–S1508. https://doi.org/10.21037/tcr.2016.12.30

Sorrentino, V., Romani, M., Mouchiroud, L., Beck, J.S., Zhang, H., D'Amico, D., Moullan, N., Potenza, F., Schmid, A.W., Rietsch, S., Counts, S.E., Auwerx, J., 2017. Enhancing mitochondrial proteostasis reduces amyloid-β proteotoxicity. Nature. https://doi.org/10.1038/nature25143

Spolski, R., Li, P., Leonard, W.J., 2018. Biology and regulation of IL-2: from molecular mechanisms to human therapy. Nat. Rev. Immunol. https://doi.org/10.1038/s41577-018-0046-y

Swadling, L., Pallett, L.J., Diniz, M.O., Baker, J.M., Amin, O.E., Stegmann, K.A., Burton, A.R., Schmidt, N.M., Jeffery-Smith, A., Zakeri, N., Suveizdyte, K., Froghi, F., Fusai, G., Rosenberg, W.M., Davidson, B.R., Schurich, A., Simon, A.K., Maini, M.K., 2020. Human Liver Memory CD8+ T Cells Use Autophagy for Tissue Residence. Cell Rep. 30, 687–698.e6.

https://doi.org/10.1016/j.celrep.2019.12.050

Tamargo-Gómez, I., Mariño, G., 2018. AMPK: Regulation of Metabolic Dynamics in the Context of Autophagy. Int. J. Mol. Sci. 19, 3812. https://doi.org/10.3390/ijms19123812

Tan, A.T., Yang, N., Lee Krishnamoorthy, T., Oei, V., Chua, A., Zhao, X., Tan, H.S., Chia, A., Le Bert, N., Low, D., Tan, H.K., Kumar, R., Irani, F.G., Ho, Z.Z., Zhang, Q., Guccione, E., Wai, L.E., Koh, S., Hwang, W., Chow, W.C., Bertoletti, A., 2019. Use of Expression Profiles of HBV-DNA Integrated Into Genomes of Hepatocellular Carcinoma Cells to Select T Cells for Immunotherapy. Gastroenterology. https://doi.org/10.1053/j.gastro.2019.01.251

Tan, H., Yang, K., Li, Y., Shaw, T.I., Wang, Y., Blanco, D.B., Wang, X., Cho, J.H., Wang, H., Rankin, S., Guy, C., Peng, J., Chi, H., 2017. Integrative Proteomics and Phosphoproteomics Profiling Reveals Dynamic Signaling Networks and Bioenergetics Pathways Underlying T Cell Activation. Immunity 46, 488–503. https://doi.org/10.1016/j.immuni.2017.02.010

Tan, J.T., Dudl, E., LeRoy, E., Murray, R., Sprent, J., Weinberg, K.I., Surh, C.D., 2001. IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proc. Natl. Acad. Sci. 98, 8732–8737. https://doi.org/10.1073/pnas.161126098

Travis, M.A., Sheppard, D., 2014. TGF-β activation and function in immunity. Annu. Rev. Immunol. https://doi.org/10.1146/annurev-immunol-032713-120257

Tsakiri, E.N., Gumeni, S., Vougas, K., Pendin, D., Papassideri, I., Daga, A., Gorgoulis, V., Juhász, G., Scorrano, L., Trougakos, I.P., 2019. Proteasome dysfunction induces excessive proteome instability and loss of mitostasis that can be mitigated by enhancing mitochondrial fusion or autophagy. Autophagy 15, 1757–1773. https://doi.org/10.1080/15548627.2019.1596477

Uchikura, K., Wada, T., Hoshino, S., Nagakawa, Y., Aiko, T., Bulkley, G.B., Klein, A.S., Sun, Z., 2004. Lipopolysaccharides induced increases in Fas ligand expression by Kupffer cells via mechanisms dependent on reactive oxygen species. Am. J. Physiol. Liver Physiol. 287, G620–G626. https://doi.org/10.1152/ajpgi.00314.2003

Van der Leek, A.P., Yanishevsky, Y., Kozyrskyj, A.L., 2017. The kynurenine pathway as a novel link between allergy and the gut microbiome. Front. Immunol. https://doi.org/10.3389/fimmu.2017.01374

van der Windt, G.J., Everts, B., Chang, C.H., Curtis, J.D., Freitas, T.C., Amiel, E., Pearce, E.J., Pearce, E.L., 2012. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 36, 68–78. https://doi.org/10.1016/j.immuni.2011.12.007

Van Der Windt, G.J.W., O'Sullivan, D., Everts, B., Huang, S.C.C., Buck, M.D., Curtis, J.D., Chang, C.H., Smith, A.M., Ai, T., Faubert, B., Jones, R.G., Pearce, E.J., Pearce, E.L., 2013. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1221740110

Vuillefroy de Silly, R., Dietrich, P.Y., Walker, P.R., 2016. Hypoxia and antitumor CD8+ T cells: An incompatible alliance? Oncoimmunology. https://doi.org/10.1080/2162402X.2016.1232236

Waggoner, S.N., Cornberg, M., Selin, L.K., Welsh, R.M., 2011. Natural killer cells act as rheostats modulating antiviral T cells. Nature 481, 394–8. https://doi.org/10.1038/nature10624

Wang, B.-J., Bao, J.-J., Wang, J.-Z., Wang, Y., Jiang, M., Xing, M.-Y., Zhang, W.-G., Qi, J.-Y., Roggendorf, M., Lu, M.-J., Yang, D.-L., 2011. Immunostaining of PD-1/PD-Ls in liver tissues of patients with hepatitis and hepatocellular carcinoma. World J. Gastroenterol. 17, 3322–9. https://doi.org/10.3748/wjg.v17.i28.3322

Wang, R., Dillon, C.P., Shi, L.Z., Milasta, S., Carter, R., Finkelstein, D., McCormick, L.L., Fitzgerald, P., Chi, H., Munger, J., Green, D.R., 2011. The Transcription Factor Myc Controls Metabolic Reprogramming upon T Lymphocyte Activation. Immunity 35, 871–882. https://doi.org/10.1016/j.immuni.2011.09.021

Wang, R., Green, D.R., 2012. Metabolic checkpoints in activated T cells. Nat. Immunol. https://doi.org/10.1038/ni.2386

Weintraub, K., 2016. Take two: Combining immunotherapy with epigenetic drugs to tackle cancer. Nat. Med. 22, 8–10. https://doi.org/10.1038/nm0116-8

Wen, Q., Han, T., Wang, Z., Jiang, S., 2020. Role and mechanism of programmed death‑ligand-1 in hypoxia‑induced liver cancer immune escape (Review). Oncol. Lett. 19, 2595–2601. https://doi.org/10.3892/ol.2020.11369

Weston, C.J., Zimmermann, H.W., Adams, D.H., 2019. The Role of Myeloid-Derived Cells in the Progression of Liver Disease. Front. Immunol. 10. https://doi.org/10.3389/fimmu.2019.00893

Wherry, E.J., Ha, S.J., Kaech, S.M., Haining, W.N., Sarkar, S., Kalia, V., Subramaniam, S., Blattman, J.N., Barber, D.L., Ahmed, R., 2007. Molecular Signature of CD8+ T Cell Exhaustion during Chronic Viral Infection. Immunity 27, 670–684. https://doi.org/10.1016/j.immuni.2007.09.006

Wherry, E.J., Kurachi, M., 2015. Molecular and cellular insights into T cell exhaustion. Nat. Rev. Immunol. https://doi.org/10.1038/nri3862

Widjaja, C.E., Olvera, J.G., Metz, P.J., Phan, A.T., Savas, J.N., De Bruin, G., Leestemaker, Y., Berkers, C.R., De Jong, A., Florea, B.I., Fisch, K., Lopez, J., Kim, S.H., Garcia, D.A., Searles, S., Bui, J.D., Chang, A.N., Yates, J.R., Goldrath, A.W., Overkleeft, H.S., Ovaa, H., Chang, J.T., 2017. Proteasome activity regulates CD8+ T lymphocyte metabolism and fate specification, in: Journal of Clinical Investigation. https://doi.org/10.1172/JCI90895

Wilson, G.K., Tennant, D.A., McKeating, J.A., 2014. Hypoxia inducible factors in liver disease and hepatocellular carcinoma: Current understanding and future directions. J. Hepatol. https://doi.org/10.1016/j.jhep.2014.08.025

Wu, W., Shi, Y., Li, S., Zhang, Y., Liu, Y., Wu, Y., Chen, Z., 2012. Blockade of Tim-3 signaling restores the virus-specific CD8 + T-cell response in patients with chronic hepatitis B. Eur. J. Immunol. https://doi.org/10.1002/eji.201141852

Xu, D.Z., Wang, X.Y., Shen, X.L., Gong, G.Z., Ren, H., Guo, L.M., Sun, A.M., Xu, M., Li, L.J., Guo, X.H., Zhen, Z., Wang, H.F., Gong, H.Y., Xu, C., Jiang, N., Pan, C., Gong, Z.J., Zhang, J.M., Shang, J., Xu, J., Xie, Q., Wu, T.F., Huang, W.X., Li, Y.G., Xu, J., Yuan, Z.H., Wang, B., Zhao, K., Wen, Y.M., 2013. Results of a phase III clinical trial with an HBsAg-HBIG immunogenic complex therapeutic vaccine for chronic hepatitis B patients: Experiences and findings. J. Hepatol. https://doi.org/10.1016/j.jhep.2013.05.003

Xu, X., Araki, K., Li, S., Han, J.-H., Ye, L., Tan, W.G., Konieczny, B.T., Bruinsma, M.W., Martinez, J., Pearce, E.L., Green, D.R., Jones, D.P., Virgin, H.W., Ahmed, R., 2014. Autophagy is essential for effector CD8+ T cell survival and memory formation. Nat. Immunol. 15, 1152–1161. https://doi.org/10.1038/ni.3025

Xu, Y., Chaudhury, A., Zhang, M., Savoldo, B., Metelitsa, L.S., Rodgers, J., Yustein, J.T., Neilson, J.R., Dotti, G., 2016. Glycolysis determines dichotomous regulation of T cell subsets in hypoxia. J. Clin. Invest. https://doi.org/10.1172/JCI85834

Yao, C., Sun, H.-W., Lacey, N.E., Ji, Y., Moseman, E.A., Shih, H.-Y., Heuston, E.F., Kirby, M., Anderson, S., Cheng, J., Khan, O., Handon, R., Reilley, J., Fioravanti, J., Hu, J., Gossa, S., Wherry, E.J., Gattinoni, L., McGavern, D.B., O'Shea, J.J., Schwartzberg, P.L., Wu, T., 2019. Single-cell RNA-seq reveals TOX as a key regulator of CD8+ T cell persistence in chronic infection. Nat. Immunol. 20, 890–901. https://doi.org/10.1038/s41590-019-0403-4

Yin, Y., Choi, S.C., Xu, Z., Perry, D.J., Seay, H., Croker, B.P., Sobel, E.S., Brusko, T.M., Morel, L., 2015. Normalization of CD4+ T cell metabolism reverses lupus. Sci. Transl. Med. https://doi.org/10.1126/scitranslmed.aaa0835

Yokosuka, T., Takamatsu, M., Kobayashi-Imanishi, W., Hashimoto-Tane, A., Azuma, M., Saito, T., 2012. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J. Exp. Med. 209, 1201–1217. https://doi.org/10.1084/jem.20112741

Yoon, S.K., Seo, Y.B., Im, S.J., Bae, S.H., Song, M.J., You, C.R., Jang, J.W., Yang, S.H., Suh, Y.S., Song, J.S., Kim, B.M., Kim, C.Y., Jeong, S.H., Sung, Y.C., 2015. Safety and immunogenicity of therapeutic DNA vaccine with antiviral drug in chronic HBV patients and its immunogenicity in mice. Liver Int. 35, 805–815. https://doi.org/10.1111/liv.12530

Zeng, Y., Li, Y., Xu, Z., Gan, W., Lu, L., Huang, X., Lin, C., 2019. Myeloid-derived suppressor cells expansion is closely associated with disease severity and progression in HBV-related acute-on-chronic liver failure. J. Med. Virol. 91, 1510–1518. https://doi.org/10.1002/jmv.25466

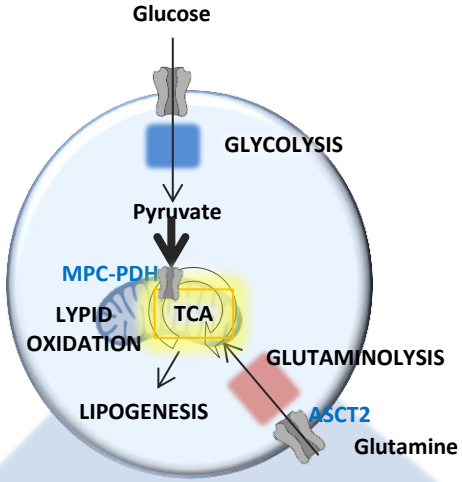
Zhang, L., Romero, P., 2018. Metabolic Control of CD8+ T Cell Fate Decisions and Antitumor Immunity. Trends Mol. Med. 24, 30–48. https://doi.org/10.1016/j.molmed.2017.11.005

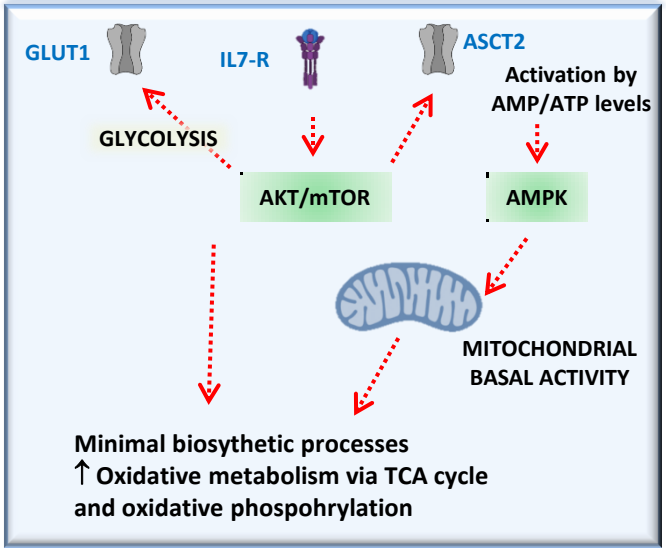
Zhang, N., Bevan, M.J., 2011. CD8+ T Cells: Foot Soldiers of the Immune System. Immunity. https://doi.org/10.1016/j.immuni.2011.07.010

Zhou, C., Li, C., Gong, G.Z., Wang, S., Zhang, J.M., Xu, D.Z., Guo, L.M., Ren, H., Xu, M., Xie, Q., Pan, C., Xu, J., Hu, Z., Geng, S., Zhou, X., Wang, X., Zhou, X., Mi, H., Zhao, G., Yu, W., Wen, Y.M., Huang, L., Wang, X.Y., Wang, B., 2017. Analysis of immunological mechanisms exerted by HBsAg-HBIG therapeutic vaccine combined with Adefovir in chronic hepatitis B patients. Hum. Vaccines Immunother. https://doi.org/10.1080/21645515.2017.1335840

Zuniga, E.I., Macal, M., Lewis, G.M., Harker, J.A., 2015. Innate and Adaptive Immune Regulation During Chronic Viral Infections. Annu. Rev. Virol. 2, 573–597. https://doi.org/10.1146/annurev-virology-100114-055226

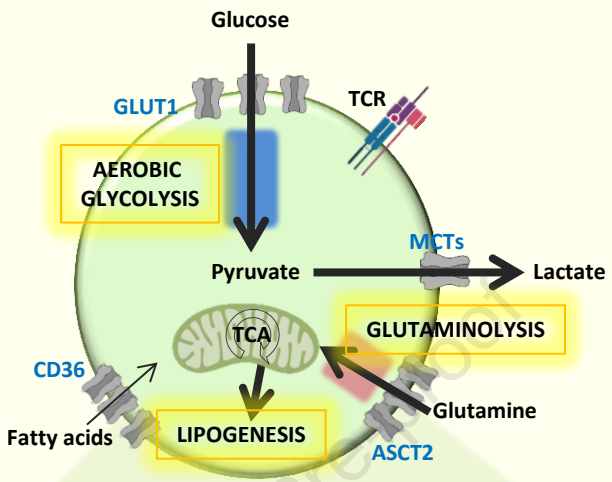
Metabolically quiescent
(low energy demand)

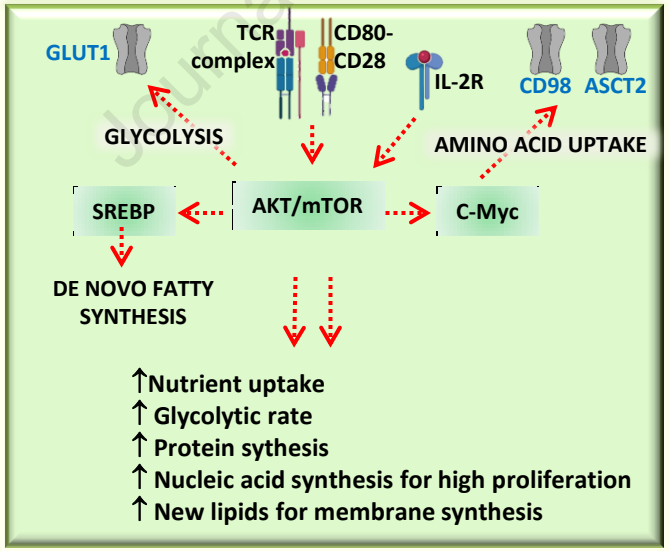




Naïve CD8 T cell

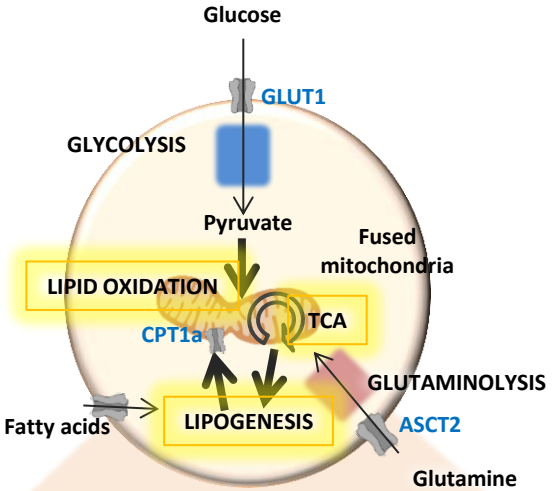
Metabolically active
(high energy demand)

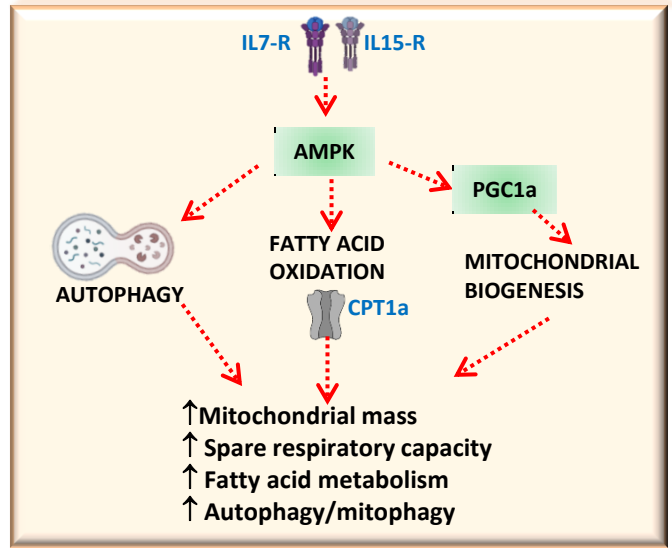




Effector CD8 T cell

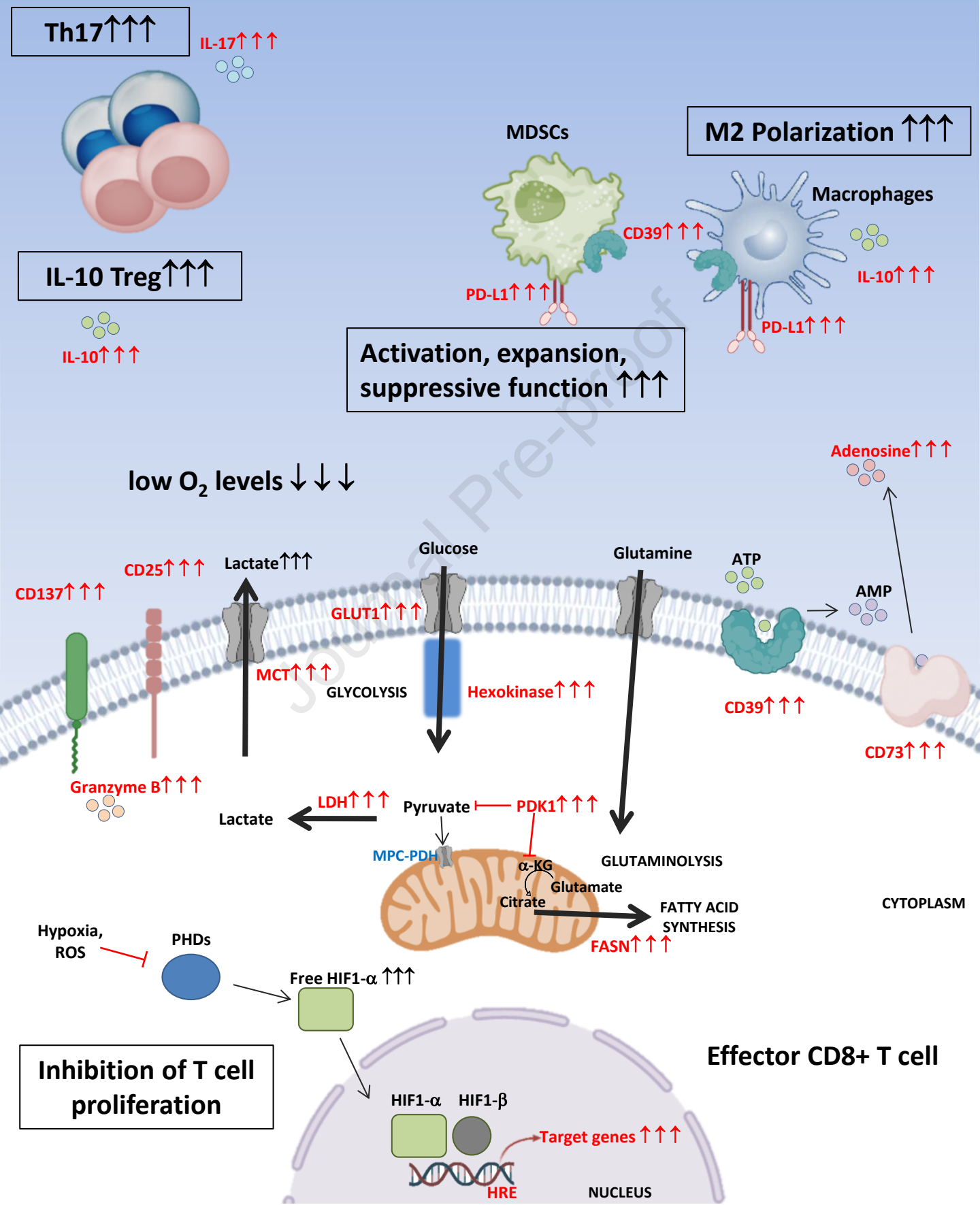
Metabolically primed
(low/intermediate energy demand)



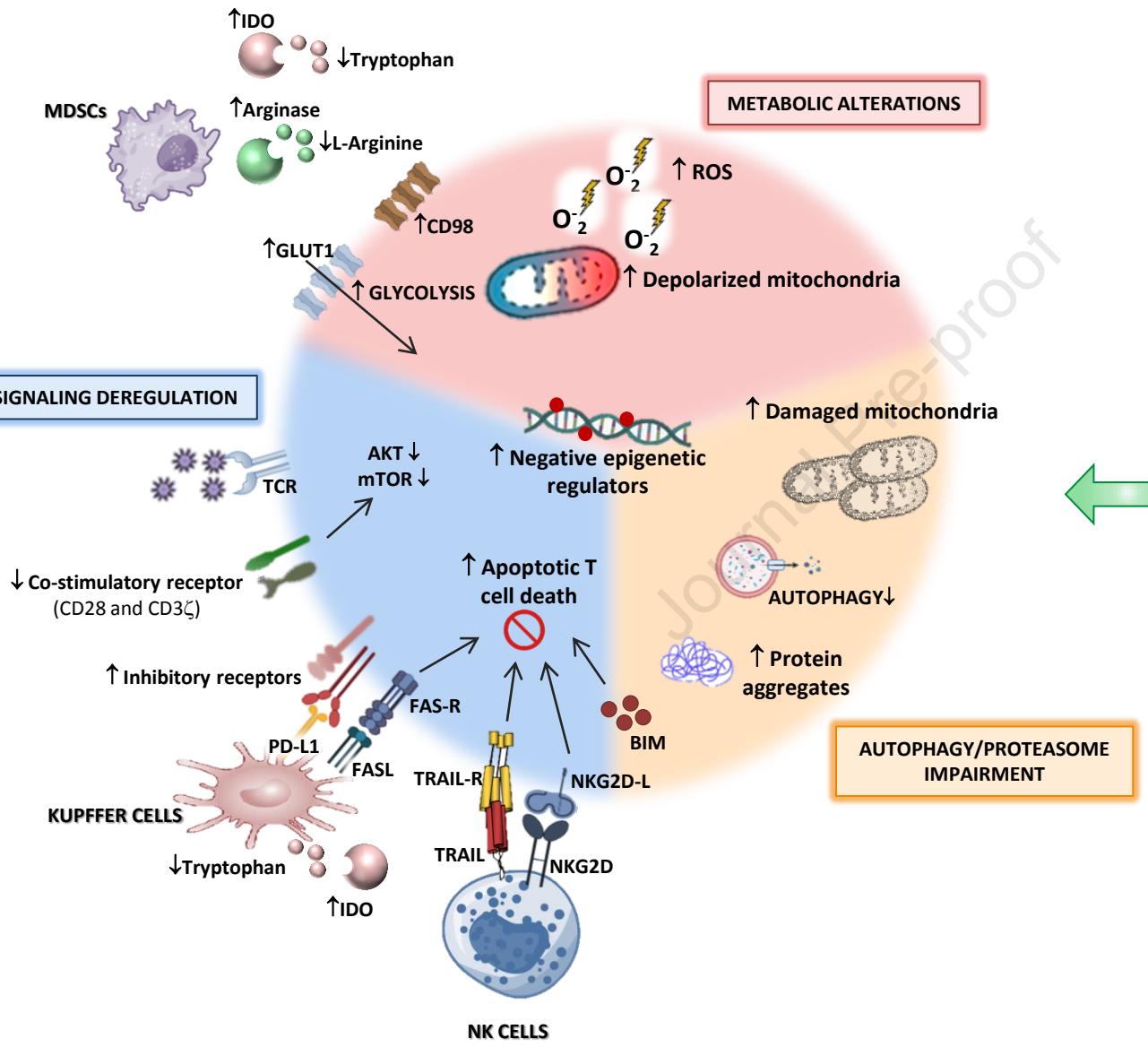


Memory CD8 T cell

Hypoxic Microenvironment and Immune Suppression



EXHAUSTED HBV CD8+ T CELLS



THERAPEUTIC APPROACHES

Immune checkpoint blockade to restore both signaling and mitochondrial deregulations

Cytokine restoration (e.g. IL-2 and IL-12) to restore signaling and metabolic deregulations

Metabolic restoration with antioxidants to restore mitochondrial dysfunctions

Proteostasis restoration with polyphenols to restore proteasome and autophagy systems

Therapeutic vaccination to restore antigen presentation and T cell activation

TCR-redirected/CAR T cells to restore both signaling and functional defects

Highlights

- HBV-specific CD8+ T cell dysfunction is a key determinant of chronic HBV persistence.
- HBV-specific CD8+ T cells express signaling, metabolic and transcriptional defects underlying inefficient antiviral functions.
- Targeting metabolic deregulations can restore *in vitro* efficient HBV-specific CD8+ T cell responses in chronic HBV infection.