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Review

The role of natural killer cells in autoimmune liver disease: A comprehensive review

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ABSTRACT

Natural Killer (NK) cells are important players of the innate arm of the immune system and provide an early defense against pathogens and tumor-transformed cells. Peripheral blood NK (PB-NK) cells were first identified because of their ability to spontaneously kill tumor-cell targets *in vitro* without the need for specific antigen priming, which is the reason that they were named 'natural killer' cells. The characterization of NK cells in human tissues and body organs represented another important step forward to better understand their physiology and physiopathology. In this regard, many reports revealed over the past decade a differential anatomic distribution of NK cell subsets in several sites such as the intestine, lung, cervix, placenta and liver as well as in secondary lymphoid organs such as spleen, lymph nodes and tonsils. Among all these tissues, the liver is certainly unique as its parenchyma contains an unusually high number of infiltrating immune cells with 30–50% of total lymphocytes being NK cells. Given the constant liver intake of non-self antigens from the gastrointestinal tract via the portal vein, hepatic NK (H-NK) cells must retain a certain degree of tolerance in the context of their immune-surveillance against dangers to the host. Indeed, the breakdown of the tolerogenic state of the liver-associated immune system has been shown to induce autoimmunity. However, the role of NK cells during the course of autoimmune liver diseases is still being debated mainly because a complete characterization of H-NK cells normally resident in healthy human liver has not yet been fully disclosed. Furthermore, the differences in phenotype and functions between human and mouse H-NK cells often preclude translation of results obtained from murine models into experimental approaches to be performed in humans. Here, we provide an extensive characterization of the phenotype of H-NK cells physiologically resident in the human liver by both mentioning data available in literature and including a set of original results recently developed in our laboratory. We then review our current knowledge in regard to the contribution of H-NK cells in regulating local immune homeostasis and tolerance as well as in inducing the development of liver autoimmunity.

1. Introduction

The liver is constantly exposed to food-derived antigens and microbes that arrive to this organ from the gastrointestinal tract via the portal vein. In this context, innate immune cells physiologically resident in the liver must constantly provide a correct defense against pathogens while maintaining tolerance to food antigens and commensal bacteria. An imbalance between these two conditions may result in the development of an inflammatory or autoimmune liver disease [1].

Natural Killer (NK) cells are important effectors of the innate immune system and in humans they constitute up to 15% of total peripheral blood lymphocytes and are also highly enriched in several tissues and organs [1,2]. NK cells are mainly involved in the defense against infections and tumors but they are also endowed with the ability to interact with other immune cells in order to orchestrate immune responses and link innate with adaptive immunity. The fact that NK cells do not require an immunological memory or a prior antigen sensitization makes them ready to fight against pathogens and malignancies in the early phases of immune responses. In fact, NK cell functions are finely controlled by a

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dynamic balance between inhibitory and activating NK cell receptors (NKRs) with the final aim to eliminate potential dangers to the host while sparing autologous healthy cells [3]. Several lines of evidence indicate that NK cells play also a key role in the maintenance of immune tolerance and that aberrancies in the NK-cell mediated immune homeostasis might lead to the onset of autoimmunity. In particular, it has been both hypothesized that NK cells might be actively involved in the pathogenesis of autoimmune diseases by either exacerbating or limiting their immune-regulatory functions, including those in response to auto-antigens such as major histocompability complex of class-I (MHC-I) molecules that normally prevent NK cell activation [4–8].

The organization of liver immune system is particularly unique as it contains an unusually high number of hepatic NK (H-NK) cells that account for up to 50% of total liver lymphocytes [1,2]. Moreover, the fact that H-NK cells have been found to be in close contact with damaged hepatocytes during the course of autoimmune hepatitis (AIH) further supports the theory that NK cells might be somewhat involved in the pathogenesis of liver autoimmunity [9].

This review focuses on the studies that have explored the role of H-NK in the regulation of liver immune tolerance and in the pathogenesis of autoimmune liver diseases. While many of these original investigations have provided important insights into the role of NK cells in liver immunological homeostasis, a great part of them have been performed in murine models, which do not always accurately describe human physiology and physiopathology given the several phenotypic and functional disparities of H-NK cells between man and mouse. Here, we highlight such differences and we also present original data showing an extensive characterization of the phenotype H-NK cells in the healthy human liver.

2. Liver immune system

The liver is the largest internal organ that receives blood through the portal vein and hepatic artery, which are supplied from the gastrointestinal tract and the aorta, respectively. Approximately 2000 L of blood passes through the liver via the portal vein daily, thus constantly exposing the liver to a large quantity of foreign antigens [10]. As such, the liver must retain a certain degree of immune tolerance to these antigens but must also be able to respond if necessary to true dangers to the host. In line with this, the liver is considered a mediator of immune tolerance based on early studies reporting successful engrafts of transplanted livers into Human leukocyte antigen (HLA) mismatched recipients from donors and the induction of systemic tolerance when the liver is simultaneously transplanted with another organ [10,11].

On the other side, the liver is also highly involved in both local and systemic inflammatory responses given its unique architecture and cellular composition. Blood entering the portal vein and hepatic artery eventually drains into portal triads, which is composed of branches of the portal vein and hepatic artery as well as bile ducts. It then passes through the sinusoidal network and exits via the central vein [12]. Endothelial cells and hepatocytes represent the vast majority of cell types in the liver, while Kupffer cells, lymphocytes, biliary cells and hepatic stellate cells comprise for a minor fraction of liver cell populations [11].

The Liver Sinusoidal Endothelial Cells (LSECs) shapes the liver sinusoids and separates the parenchyma from components passing through the bloodstream. LSECs contain fenestrae, which allow the entrance of small or flexible molecules from the blood into liver parenchyma. Underneath the LSEC layer is the Space of Disse, which harbors an extracellular matrix allowing for the control of sinusoidal blood flow. LSEC themselves are unique endothelial cells endowed with ability to operate as antigen presenting cells (APCs) able to *i)* recognize dangerous non-self antigens through the expression of pattern recognition receptors (PRRs), *ii)* to present antigen to $CD4^{pos}$ T cells, *iii)* to perform antigen cross-antigen presentation to $CD8^{pos}$ T cells and *iv)* to also establish naïve T cell tolerance [10].

Protruding from the LSEC fenestrae are Kupffer cells, which are interdispersed throughout the sinusoid [12]. As resident liver macrophages highly active in phagocytosis, Kupffer cells play a crucial role in liver immune surveillance [10–12]. Through the expression of a multitude of innate immune receptors, and in particular PRRs such as Toll-Like Receptors (TLRs), Kupffer cells can recognize bacterial and viral products. After binding pathogens, these liver macrophages act as regulatory cells that can either amplify or dampen immune responses by producing and secreting cytokines and chemokines such as IL-6, tumor necrosis factor α (TNFα), IL-12, IL-18, IL-15, IL-10, CCL3/MIP1a and CCL5/RANTES [12–15].

3. Liver and autoimmunity

Autoimmune diseases affecting the liver are mainly represented by AIH, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). They are all chronic disorders with fluctuating clinical courses alternating relapses and quiescence (Table 1) [16].

AIH is characterized by an immune-mediated hepatocellular necrosis and inflammation, leading to progressive destruction of the hepatic parenchyma, which often results in liver cirrhosis and a

Table 1
Differential features of autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

	AIH	PBC	PSC
Gender	70% ♀/30% ♂	>90% ♀/<10% ♂	30% ♀/70% ♂
Age at onset	3rd–5th decade	5th decade	3rd–4th decade
Prevalence in developed countries	1:5000–1:10000	1:5000–1:10000	1:10000
Laboratory findings	AST over AP Hyper-γ-globulinemia (mostly IgG)	AP over AST Hyper-γ-globulinemia (mostly IgM) Circulating ICs	AP over AST Hyper-γ-globulinemia (IgG and/or IgM)
Autoantibodies	ANA, SMA (Type I AIH); LKM (Type II AIH); SLA/LP (Type 3 AIH)	AMA (M2)	p-ANCA
Liver histopatholohy	Interface hepatitis	Patchy destruction of interlobular bile ducts, patchy destruction of interlobular bile ducts, patchy destruction of interlobular bile ducts	Portal tract inflammation with infiltration of lymphocytes in the bile ducts and ductural proliferation
Associated HLA-Alleles	HLA-DR3	HLA-DR8	HLA-DR3
References	16, 17–23	16, 24–31	16, 32–38

♀: female; ♂: male; AST: aspartate aminotransferase; AP: alkaline phosphatase; ICs: Immune Complexes; ANA: anti-nuclear antibodies; SMA: anti-smooth muscle antibodies; LKM: anti-liver kidney microsomal antibodies; SLA/LP: anti-soluble liver or liver-pancreas antigens antibodies; AMA: anti-mitochondrial antibodies; p-ANCA: anti-neutrophil cytoplasmic antibodies, perinuclear pattern; HLA: human leukocyte antigens.

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