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Review

Natural killer and natural killer T cells in liver fibrosis ☆

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ABSTRACT

The liver lymphocyte population is enriched with natural killer (NK) cells, which play a key role in host defense against viral infection and tumor transformation. Recent evidence from animal models suggests that NK cells also play an important role in inhibiting liver fibrosis by selectively killing early or senescence activated hepatic stellate cells (HSCs) and by producing the anti-fibrotic cytokine IFN-γ. Furthermore, clinical studies have revealed that human NK cells can kill primary human HSCs and that the ability of NK cells from HCV patients to kill HSCs is enhanced and correlates inversely with the stages of liver fibrosis. IFN-α treatment enhances, while other factors (e.g., alcohol, TGF-β) attenuate, the cytotoxicity of NK cells against HSCs, thereby differentially regulating liver fibrogenesis. In addition, the mouse liver lymphocyte population is also enriched for natural killer T (NKT) cells, whereas human liver lymphocytes have a much lower percentage of NKT cells. Many studies suggest that NKT cells promote liver fibrogenesis by producing pro-fibrotic cytokines such as IL-4, IL-13, hedgehog ligands, and osteopontin; however, NKT cells may also attenuate liver fibrosis under certain conditions by killing HSCs and by producing IFN-γ. Finally, the potential for NK and NKT cells to be used as therapeutic targets for anti-fibrotic therapy is discussed. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

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1. Introduction

Liver fibrosis and its end-stage consequence, cirrhosis, represent the final common pathway of virtually all chronic liver diseases and affect hundreds of millions of people worldwide. Despite impressive advancements in the field, no treatment options currently exist to cure fibrosis, with the exception of liver transplantation. The complex mechanisms driving the progression of chronic liver injury to fibrosis are not fully understood because of the multifaceted and often paradoxical intercellular relationships. Accumulating evidence suggests that hepatic stellate cell (HSC) activation during liver injury is a key step in the development of liver fibrosis [1–5]. In a healthy liver, HSCs are quiescent and play a central role in the storage of retinol (vitamin A compound). After injury, HSCs become activated and transdifferentiate into matrix-producing cells termed myofibroblasts. The activation of HSCs is controlled by many types of cytokines, growth factors, immune cells, and other factors [1–5]. Among the immune cells involved, macrophages have been shown not only to contribute to the pathogenesis of liver fibrosis but also to promote liver fibrosis resolution [6]. Dendritic cells (DCs) were reported to exacerbate liver fibrosis via the production of TNF-α by an early paper [7], but a recent study using more specific DC markers suggests that DCs ameliorate liver fibrosis by promoting fibrosis regression via the secretion of matrix metalloproteinase-9 [8]. Additionally, the functions of natural killer (NK) and NKT cells in liver fibrogenesis have recently received great attention because these cells are enriched among liver lymphocytes and are also markedly altered in various liver diseases [9]. In this review, we highlight recent advances in the understanding of the functions of NK and NKT cells that are important for the pathogenesis of liver fibrogenesis and will briefly discuss NK and NKT cells as potential therapeutic targets for anti-fibrotic therapy.

2. NK and NKT cell biology

NK cells are lymphocytes of the innate immune system that recognize and kill infected and tumorigenic cells. These cells represent a third lineage of lymphoid cells that is distinct from T and B cells. Unlike B and T cells, NK cells do not express an antigen receptor. Instead, they rely upon an array of cell surface receptors to detect changes in the expression of host cell surface molecules that typically appear on a variety of 'stressed cells', including microbe- or virus-infected cells, transformed cells, and injured cells [10]. The decision to kill a cell is made based on the net balance of signals delivered by

Abbreviations: α-GalCer, α-galactosylceramide; DC, Dendritic cell; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; HSC, hepatic stellate cell; iNKT, invariant NKT; KIR, killer Ig-like receptor; MCD diet, methionine choline-deficient diet; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NK cell, natural killer cell; NKT cell, natural killer T cell; RAE-1, retinoic acid inducible gene 1

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inhibitory and activating receptor molecules that are expressed on NK cells. The inhibitory receptors include killer Ig-like receptors (KIRs) and Ly-49A and CD94/NKG2 receptors that recognize MHC class I molecules (inhibitory ligands) expressed on nearly all normal cells, which subsequently inactivate NK cell function. Thus, NK cells do not kill normal host cells. The stimulatory receptors present on NK cells include NKp46, NKp30 and NKp44, which are collectively referred to as natural cytotoxicity receptors, NKG2D, and DNAX accessory molecule-1 (CD226) [10–12]. Among these, NKG2D is the most well-defined receptor, which binds its ligands (e.g., MICA/B and ULBP in humans, RAE-1 and MULT1 in mice) that are expressed on target cells and subsequently promotes NK cell activation. After activation, NK cells can directly kill target cells via the exocytosis of perforin- and granzyme-containing cytoplasmic granules. NK cells can also kill target cells in a perforin-independent manner by utilizing FAS ligand, TNF-α, and TNF-related apoptosis-inducing ligands (TRAIL). Additionally, production of many cytokines (particularly IFN-γ, TNF-α, IL-10, IL-22) and chemokines (including MIP-1α and -β and RANTES) is another important mechanism by which NK cells regulate target cells and immune responses [13,14].

NKT cells are a heterogeneous group of T lymphocytes that recognize lipid antigens presented by the nonclassical MHC class I-like molecule CD1 [15]. Human tissues express five distinct isoforms of CD1, including CD1a, -b, -c, -d, and -e, whereas mice only express CD1d. The CD1d-dependent NKT cells can be grouped into two types of cells: type I and type II NKT cells. Type I NKT cells, which are also called classical or invariant NKT (iNKT) cells because they express an invariant T cell receptor α (TCR-α) chain, comprise 95% of liver NKT cells. Type II NKT cells express diverse TCRs and make up less than 5% of liver NKT cells. Upon activation by lipid antigens such as α-galactosylceramide (α-GalCer), iNKT cells are able to produce large quantities of IFN-γ, IL-4, IL-13, TNF-α, IL-17, and many other cytokines. Activated iNKT cells also produce cytotoxic mediators such as perforin, Fas ligand, and TRAIL to kill target cells. Although activation of iNKT cells by the exogenous lipid antigen α-GalCer has been extensively investigated, the endogenous ligands that activate NKT cells remain largely unknown.

3. Hepatic NK and NKT cells

NK cells in the liver, which were originally called “Pit” cells in rats, are located in the hepatic sinusoids in close vicinity to liver nonparenchymal cells and represent a unique organ-associated NK cell population [16]. Under normal physiological conditions, hepatic NK cells have a rapid turnover, with an estimated residence time of 1 to 2 weeks in the liver. Because their proliferative activity is very low, the hepatic NK cell population is continuously replenished from an extra-hepatic source of stem cells, which is most likely located in the bone marrow [16]. The frequency of NK cells among liver lymphocytes is much higher in rats and humans than that in mice; in mice, 10% of hepatic lymphocytes are NK cells, while 30–50% of liver lymphocytes from rat and human livers are NK cells [9]. Various pathological conditions (such as viral infection, acute and chronic inflammation) as well as treatments with biological response modifiers that activate the immune system (such as polyinosinic–polycytidylic acid [poly I:C] or IFNs) significantly increase the number of NK cells in the liver (see review [9] and reference therein). At present, the mechanisms underlying the enrichment of NK cells in the liver are not fully understood, although it is believed that adhesion to sinusoidal endothelial cells is an important step in the recruitment of NK cells from the vascular compartment into the liver [17]. Such adhesion is regulated by cell adhesion molecules, which mediate cell-to-cell and cell-to-matrix interactions. In humans, several cell adhesion molecules, such as CD11a/CD18, CD2, CD54, CD56, and CD58, have been detected on the surface of NK cells [18], and blocking these molecules with neutralizing antibodies markedly decreases the number of NK cells in the liver [17].

After migrating into the liver, peripheral NK cells develop into liver-specific NK cells with unique features, such as higher levels of cytotoxicity against different tumor target cells, compared to NK cells from other organs [19,20]. Such liver-specific NK cell development is believed to be controlled by the liver sinusoidal microenvironment. For example, human NK cells extracted from normal donor liver perfusates from a living donor liver transplantation were able to kill human hepatocellular carcinoma cell line HepG2 cells, an effect which was further enhanced by IL-2 treatment; however, NK cells from recipient livers with cirrhosis showed impaired anti-tumor activity even in the presence of IL-2 stimulation [19]. At present, how liver microenvironment affects liver-specific NK cell development remains unclear.

In addition to NK cells, NKT cells are also enriched among liver lymphocytes, as approximately 30–35% of mouse liver lymphocytes are NKT cells, and 5–10% of rat and human liver lymphocytes are NKT cells, which are significantly larger frequencies than that observed for peripheral blood lymphocytes (<5% NKT cells) [9]. NKT cells not only directly kill target cells but also produce a wide variety of cytokines, thereby playing diverse roles in controlling liver injury, fibrosis, regeneration, and hepatocarcinogenesis [9].

4. Anti-fibrotic effect of NK cells

NK cell killing of activated HSCs was first demonstrated by two different groups using mouse models in 2006 [21,22], and this observation was later confirmed by many additional studies both in animal models [23–32] and patients [28,31,33–36] (Table 1). Collectively, these findings suggest that NK cells selectively kill early activated (transitional) or senescence-activated HSCs but do not kill quiescent or fully activated (myofibroblasts) HSCs (Fig. 1). In addition, NK cells also produce IFN-γ, which then induces HSC apoptosis and cell cycle arrest and subsequently inhibits liver fibrosis (Fig. 1).

4.1. NK cells selectively kill early activated but not quiescent or fully activated HSCs: In vivo and in vitro evidence

In a healthy liver, HSCs are quiescent and store retinol, but in response to liver injury, they are activated and converted into highly proliferative, contractile myofibroblast-like cells [3]. Activation of HSCs in an injured liver gives rise to an array of cells at different stages of activation and trans-differentiation that display transitional phenotypes, gene expression, and functions, which can be identified by several markers. Among these, Desmin, an intermediate filament typical of contractile cells, has been widely used as the “gold standard” for identifying all stages of HSCs in rodent livers, although its expression in humans is unreliable [3]. As illustrated in Fig. 2A, immunostaining with Desmin shows numerous processes in the quiescent HSCs, while the early activated HSCs in the fibrotic mouse livers induced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) are identified as small round cells with a scant cytoplasm that lack the processes. The myofibroblast-like cells are revealed as elongated shape and localized in the portal fibrotic areas.

Immunohistochemistry analyses have shown in vivo that NK cells kill early activated, but not quiescent or fully activated, HSCs. First, the number of early activated desmin positive HSCs with an oval shape was significantly decreased in DDC-fed mice after administration of the NK cell activator poly I:C (Radaeva and Gao, unpublished data). Second, immunohistochemistry analyses show that early activated HSCs and NK cells have similar distributions throughout zones II and III of the liver parenchyma but do not reside in the periportal fibrotic area (Fig. 2B–C). Third, the direct contact between NK cells and early activated HSCs is often observed in the injured liver (Fig. 2D).

In vitro cell co-culture and cytotoxicity assays clearly demonstrate that NK cells kill early activated, but not quiescent or fully activated, HSCs (Fig. 1) [24]. Quiescent HSCs are spontaneously activated

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