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Review

Immunotherapeutic modulation of the suppressive liver and tumor microenvironments

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ABSTRACT

The liver is an immunologically unique organ, consisting of resident hematopoietic and parenchymal cells which often contribute to a relatively tolerant microenvironment. It is also becoming increasingly clear that tumor-induced immunosuppression occurs via many of the same cellular mechanisms which contribute to the tolerogenic liver microenvironment. Myeloid cells, consisting of dendritic cells (DC), macrophages and myeloid derived suppressor cells (MDSC), have been implicated in providing a tolerogenic liver environment and immune dysfunction within the tumor microenvironment which can favor tumor progression. As we increase our understanding of the biological mechanisms involved for each phenotypic and/or functionally distinct leukocyte subset, immunotherapeutic strategies can be developed to overcome the inherent barriers to the development of improved strategies for the treatment of liver disease and tumors. In this review, we discuss the principal myeloid cell-based contributions to immunosuppression that are shared between the liver and tumor microenvironments. We further highlight immune-based strategies shown to modulate immunoregulatory cells within each microenvironment and enhance anti-tumor responses.

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Contents

1. Introduction

The liver is an immunologically unique microenvironment constantly exposed to various antigens such as microbial products from intestinal bacteria. As such, there are numerous cellular and

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molecular components that are involved with maintaining a tolerogenic liver microenvironment, yet which still endow this organ with the necessary capabilities for the development of immune responses [1]. The capability of inducing tolerance is beneficial in specific situations such as allogeneic transplantation, although opportunistic infections such as hepatitis B and other malignancies may exploit this situation and result in chronic disease. The liver contains a different cellular distribution of lymphocytes, such as the higher proportion of NK and NKT cells compared to other lymphoid organs such as the spleen. DC and macrophages present within the liver are primarily responsible for antigen presentation, although non-lymphoid hepatocytes and liver sinusoidal endothelial cells also have limited antigen presentation capabilities.

2. Resident Kupffer cells and macrophages contribute to an immunosuppressive liver microenvironment

Kupffer cells (KC), identified based upon CD68 (microsialin) expression and as a subset of $CD11b^+/F4/80^+$ cells, are the largest group of tissue resident macrophages located in the liver and lie within the periportal area of the hepatic sinusoids. A major function of KC is the phagocytosis of particulates, apoptotic cells and microorganisms present within the portal circulation [1]. KC have APC functions with antigen uptake and processing capabilities and express low levels of MHC class II and co-stimulatory molecules at a steady state. Upon encounter with an antigen, KC can release a variety of reactive oxygen species (superoxide anions, hydrogen peroxide and nitric oxide) as well as pro-inflammatory cytokines such as TNFα, IL-1 and IL-6. However, KC have been shown to induce tolerance in models of liver allografts and tolerance to soluble antigens encountered within the circulation [2–4]. The implicated tolerogenic mechanisms have included expression of immunoregulatory cytokines/modulators such as IL-10, TGF-β and IDO (indolamine 2,3 dioxygenase), NO and Fas [5,6]. However, a recent study has also implicated the abundant production of prostaglandins such as PGE_2 and 15-deoxy-delta12, 14-PGJ_2 (15d-PGJ_2), that lead to T cell suppression [3]. In addition, the expression of the regulatory co-stimulatory molecule, B7-H1 (PD-L1) on KC has also been implicated in reducing the inflammation induced in a partial liver warm ischemia/reperfusion model system [7], whereas stimulation via the PD-L1/PD-1 axis can be detrimental in a malignant setting such as human hepatocellular carcinoma [8].

3. Contribution of dendritic cells towards a tolerogenic liver microenvironment

Multiple subsets of hepatic DC are present within the liver consisting of conventional DC, herein referred to as DC ($CD11c^+$ MHC class II^+ $CD11b^+$ or $CD8\alpha^+$) and pDC ($CD11c^{low}$;$B220^+$) [9–12], as well as the controversial NKDC subset that has been noted by some groups [13]. The major DC subset is the pDC, which can make up more than 50% of the DC present in this organ. Liver DC are strategically situated around the portal tracts to capture exogenous antigens. Previous studies involving characterization of the entire liver DC populations have shown reduced expression of co-stimulatory molecules and reduced production of pro-inflammatory cytokines, often in reference to an immature state and resulting in lower allogeneic immunostimulatory properties in mixed lymphocyte reactions compared to their splenic counterparts [11,14]. However, detailed analyses of specific subsets have shown there are drastic biological activities within the heterogenous DC population. Hepatic DC can cross-present antigen to induce activation and proliferation of $CD8^+$ T cells in the liver, in a DC-dependent manner, as transient ablation of DC with diphtheria toxin in CD11c-GFP-diphtheria toxin receptor (DTR; [15]) mice dramatically reduced OT-I T cell proliferation [16]. One report revealed $CD11c^+CD11b^+CD8\alpha^-$ and $CD11c^+CD11b^{low}CD8\alpha^+$ DC had comparable allostimulatory properties and pro-inflammatory cytokine production similar to their splenic counterparts while the pDC population resulted in minimal T cell proliferation and cytokine production [14]. The authors concluded the difference between the liver and spleen is the greater degree of pDC present in the liver and the overall relative paucity of the cDC present, which is reversed in the spleen. Further confirmation was obtained with human liver DC demonstrating lower allo-proliferation and T cell hypo-responsiveness following restimulation and a higher propensity to induce Tregs [17]. However, it has also been demonstrated that there are some inherent differences in liver cDC such as the expression of IL-10 and IL-27 compared to splenic DC, which have higher IL-12 production [18]. Damage to the liver results in an inflammatory response and chronic inflammation leading to liver fibrosis was dependent on DC-produced TNF, resulting in increased T cell proliferation and NK cell activation [19]. Dependent upon the stimulus, the sterile inflammatory process of liver ischemia/reperfusion injury induced IL-10 production by DC to inhibit the action of CCR2-recruited inflammatory monocytes to the liver, thereby reducing IL-6, TNF and reactive oxygen species production and minimizing hepatic injury [20,21]. In addition, liver DC displayed decreased expression levels of Toll-like receptor (TLR)-4 resulting in reduced cytokine expression upon exposure to LPS [22]. The reduced expression of TLR4 may be strategically based upon the constant exposure to microbial products that the liver receives. When exposed to high levels of LPS beyond normal physiological levels (≥100 ng/ml), the allogeneic C3H/HeJ T cell response was partially restored to the proliferative response of splenic DC and increased the production of Th1 cytokines by T cells [22]. However, stimulation of liver DC with anti-CD40 resulted in comparable allogeneic T cell proliferative response as seen with the spleen. Furthermore, the exposure of hepatic DC to the LPS endotoxin induced a “cross-tolerance” effect by attenuating IL-12 production in CpG stimulated DC [23].

The increased frequency of pDC in the liver may also contribute to the tolerogenic microenvironment, as these cells have been shown to play a role in regulating adaptive immunity in the liver [9,11]. Although pDC are potent type I IFN producing cells that can initiate T cell responses [24–26], studies analyzing the liver DC subsets in mice and humans have demonstrated that liver pDC are responsible for T cell hypo-responsiveness [14,17]. Potential mechanisms for this include the increased production of IL-10 by pDC, an inherent biological preference towards non-Th1 T cell polarizing environment and enhanced proliferation of Tregs [27]. In vitro studies of hepatic pDC supplemented with exogenous IL-12 or neutralizing anti-IL-10 antibody improved the ability of Flt3L-expanded hepatic pDC to stimulate T cell proliferation, to levels similar to splenic pDC. Furthermore, the intrinsic biology of hepatic pDCs reveal some functional differences between their splenic and DC counterpart such as a decreased Delta4/Jagged1 Notch ligand ratio further promoting a Th2 type T cell response [27] and a higher expression of the nucleotide-binding oligomerization domain (NOD)2 [28]. In mice injected with muramyl dipeptide (MDP), a bacterial peptidoglycan, a selective increase in the expression of the negative TLR-signaling regulator, interferon regulatory factor 4 (IRF-4), and B7-H1 was observed [28]. The authors also demonstrated decreased IFNα serum levels upon CpG administration to MDP-treated mice. However, it is also worth noting that hepatic pDC produce less type I IFNs compared to splenic pDCs [28]. Further supporting the tolerogenic nature of hepatic pDC, Goubier et al. demonstrated liver pDCs mediated oral tolerance to 2,4-dinitrofluorobenzene [DNFB] and ovalbumin (OVA) antigen resulting in $CD8^+$ T cell tolerance in a $CD4^+$ T cell independent manner, thereby preventing T cell mediated contact hypersensitivity involved with ear/footpad swelling and rapidly inducing antigen specific T cell anergy or deletion [29]. Depletion of pDC using mAbs such as Gr-1 and 120G8 restored the cell-mediated DTH response.