REVIEW

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Dendritic cells and hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) represents a major health burden in the modern world. Because current treatment options for HCC are capable of providing good survival rates to only a limited number of patients, new therapeutic opportunities should be looked upon. The particularities of dendritic cells (DC) populations existing in the liver, and their consecutive selective activation of certain immunotolerant T-cell subgroups, account for the high success rate of allogeneic hepatic transplantation, currently the most efficient treatment for HCC. The particularities of dendritic cells (DCs) populations existing in the liver, and their consecutive selective activation of certain immunotolerant T-cell subgroups, account for the high success rate of allogeneic hepatic transplantation for HCC. These molecular mechanisms also open new paths towards cancer preventing and cancer curative vaccines, as well as successful immunotherapy. Our aim was to summarize the main aspects of the biology of DCs populations, especially those present in the liver, and to draw attention to their current and future roles in the curative treatment of hepatocarcinoma.

Keywords: hepatocellular carcinoma, dendritic cells, liver dendritic cells, cancer vaccines, immunotherapy.

Introduction

Hepatocellular carcinoma (HCC) ranks fifth as the most common cancer in the world with a poor prognosis and limited survival in the majority of the patients, being the third leading cause of cancer death [1, 2]. Current treatment options for HHC include surgical approaches (radical or palliative resection and liver transplantation), locoregional ablative techniques (percutaneous alcohol injection and radiofrequency ablation), interventional treatments (transcatheter arterial chemoembolization, pre-operative portal vein embolization, transcatheter arterial radioembolization) and molecular targeted therapies such as Sorafenib [3, 4]. Even though the five-year survival rate after liver transplantation is greater than 70% for HHC patients within the Milan criteria [5], only few patients benefit for this therapeutics procedure because of the low number of donors [4]. Liver resection requires a strict selection of appropriate candidates, therefore, resectability rate in HHC patients is very low [6], while the long-term survival of patients who benefited from interventional procedures is not fully satisfactory [7]. The major obstacle to improving post-treatment prognosis remains the high rate of recurrence. As a result, the developing of new efficient strategies able to improve HCC evolution is still required [8]. Although immuno-therapy is not recommended for the clinical management of HCC patients by current guidelines [4], several different immunotherapy strategies have been investigated in the last decade [9]. Various researches have identified the dendritic cells (DCs) as one of the key elements in the immune regulation, studies focusing on their role in anti-tumor immunity induction and, subsequently in cancer therapy [10, 11]. DCs are highly potent professional antigen presenting cells (APCs) that play a key role in primary immune responses, tolerance and maintenance of immune homeostasis. Additionally, they may be an important bridge between the innate and adaptive immune systems [12, 13].

The purpose of this review is to summarize the biology and function of DCs, with a special emphasis on liver DCs, and draw attention to their current and future roles in HHC immunotherapies.

Dendritic cells: a heterogeneous cells population

General characteristics

The DCs are a heterogeneous cells population derived from a bone marrow progenitor, characterized by an important plasticity, especially phenotypical but also morphological and functional. DCs heterogeneity is reflected in precursor populations, anatomical localization, different functions and the final outcome of immune response [10, 11]. Since the first type of DC was identified by Paul Langerhans in the epidermal layer of the skin in 1868, for more than a century their function has been ignored. Although Langerhans believed they were nerve

cells, the precision of his description and picture, using a primitive light microscope, are comparable with current modern imaging techniques [14]. Thus, an important link in the immune system was missing until 1973 when and Ralph M. Steinman and Zanvil A. Cohn found an unknown stellate cell population in the mouse spleen, with distinct features and the possibility of distinct functions. The term DC was proposed for this novel cell population, which also marked the moment for the start of new extensive research on DCs [15–17].

There is increasing interest in DCs development pathways and stages such as maturation, migration and homeostasis, as well as their role in immune function and immunotherapy. However, difficulties and controversies arising from the study of DCs remain and are partially explained by the DCs heterogeneity *in vivo*, their rarity in blood, differences in human DC biology and that of mice, and different methodologies used in culture conditions. Additionally, DCs are not an individualized line and are studied especially *in vitro* [18, 19].

Whilst it is well known that DCs originate from the bone marrow progenitors cells, the nature of immediate DC precursors in most tissues either remain unclear or is still an area of debate. Recent studies have attempted to shed light on the lineage origin of DCs, suggesting that there are different developmental pathways depending on progenitors and intermediate stages, cytokine, surface marker expression and functions [20].

Two DC functional states are described, as immature or mature DCs, and several factors can induce DCs maturation. Immature DCs are characterized by high phagocytic capacity, active endocytosis for certain particles and proteins, abundant major histocompatibility complex (MHC) class II products within endosomal and lysosomal compartments, low MHC surface expression, low or absent adhesive and co-stimulatory molecules, and low T-cell activation potential. Immature DCs play a major role in tolerance induction. Mature DCs are specialized APCs, which express high levels of surface MHC I and MHC II class, as well as the appropriate co-stimulatory molecules and adhesive molecules required for T-cell activation [21–23].

DCs subtypes and distribution

DCs functional anatomical classification was based on the fact that DCs function is linked to their location [24]. The two main categories of DCs subtypes present in the steady state include conventional DCs and plasmacytoid DCs, both originated from hematopoietic stem cells (HSCs) in the bone marrow *via* intermediate progenitors. Conventional DCs derived from common dendritic progenitors (CDPs) and pre-DCs, includes migratory and lymphoid-resident DC subtypes [24, 25]. Based on the precursor populations from which the various DC subsets originate, Kushwah and Hu classifies DCs derived from CDPs and pre-DC populations as conventional DCs, which includes both migratory and lymphoid-resident DC subsets, and monocyte-derived DCs and plasmacytoid DCs as non-conventional DCs [26].

Conventional DCs

Both migratory DCs and lymphoid resident DCs act in the maintenance of self-tolerance and the induction of specific immune responses. However, the function and the life history of resident DCs are restricted to one lymphoid organ [27, 28].

Migratory DCs act as sentinels acquiring the antigen or peripheral self-antigen in peripheral tissue such as the skin, lungs, pancreas, heart and gut and also in filtering sites, such as the liver and the kidney. They migrate *via* the afferent lymphatic system to eventually drain into lymph nodes where present antigens to T-cells [25, 27]. The most studied migratory DCs are epidermal Langerhans cells (LCs) and interstitial dermal DCs [25, 29].

Lymphoid resident DCs have been extensively studied in mice, where they are found to reside in lymphoid organs such as the thymus, the spleen and lymph nodes [25, 26]. Major populations of DCs in the murine lymphoid organs are CD8+ DCs and CD8- DCs that can be further divided into CD4 DCs and double/ triple negative DCs [26, 29]. The CD8+ and CD8- DCs subsets are different in phenotype, function and localization in lymphoid organs [25, 26]. Functional specialization of major murine subsets has been observed. Thus, CD8+ DCs are superior to CD8- DCs concerning exogenous antigens presentation of MHC class I molecules, a process known as cross-presentation [30]. On the other hand, several comparative transcriptional and functional studies have shown that human blood BDCA3+ DCs are homologous to murine CD8+ DCs. Given the fact that antigen cross-presentation *in vivo* occurs in secondary lymphoid organs and not in the blood, a recent study showed that all freshly isolated lymphoid organ-resident human DCs (resident BDCA1+ DCs and BDCA3+ DCs, and plasmacytoid DCs) have high intrinsic cross-presentation capacity [31].

Plasmacytoid DCs (pDCs) are a particular subset of DCs that originate in the bone marrow from CDPs which express cytokine receptors FLT3 (CD135) and M-CSF (CD115), and low levels of c-kit (CD117) [32, 33]. Unlike cDCs enter the lymphoid organs *via* afferent lymphatics, pDCs circulate through the body *via* the blood stream, and migrate into the T-cell areas of lymph nodes and spleen, mucosa-associated lymphoid tissues, thymus and liver *via* high endothelial venules [32, 34]. Whereas immature pDCs are just tolerogenic, mature pDCs have both immunogenic and tolerogenic capacities depending on its activation environment [35]. Immature pDCs express low levels of MHC class II [31]. Upon stimulation and subsequent activation, pDCs secrete numerous cytokines such as interferons (IFNs), interleukins (IL), tumor necrosis factor (TNF)-alpha and chemokines (*i.e.*, MIG, IP-10, MIP-1α, MIP-1β and RANTES) [10, 34, 36].

However, one of the most important functions of pDCs remains the rapid production of high amounts of type I IFN (IFN-I), especially in response to virus-derived nucleic acids through activation of Toll-like receptors (TLRs), both TLRs7 and 9 [25, 33]. Human pDC are known to express TLR7, TLR8, and TLR9, while murine pDCs express only TLR7 and 9 [34]. In addition, IFN-I produced by pDC induces differentiation and maturation of myeloid DCs, promotes T-cell activation and increases activity of natural killer (NK) cells [34, 37]. Through IFN-I and IL-6, pDCs induce differentiation of antibody-secreting plasma cells from activated B-cells [38], while chemokines can attract activated CD4+ and CD8+ T-cells to sites

of inflammation [37, 39]. As such, pDCs play a key role in both innate and adaptive immune responses [40].

Brief overview of liver DCs immunobiology

The liver is an important mediator of innate and adaptive immunity, and an important site of immune regulation [41, 42]. Hepatic resident cells play a key role in local immune regulation not only by their APCs function but also through their role in the recruitment of leukocyte populations [43]. As such, the liver contains a diversity of immunologically active cell types including DCs as well as other potent APCs and a diverse lymphocyte population [44, 45]. Other liver APCs are represented by liver sinusoidal endothelial cells (LSEC) which reside near the sinusoid capillaries [46, 47], Kupffer cells (KC) known as the resident liver macrophages able of moving freely in the portal system and which were found to function in conjunction with LSECs in response to antigens [48], hepatic stellate cells (HSC) which reside in the subendothelial space of Disse [42] and act as APCs for CD4 and CD8 T-cells [49] and finally, hepatocytes [45], which have been demonstrated to exert antigen presenting properties in certain conditions (Figure 1). The liver also contains diverse lymphocytes, including T-cells, natural killer (NK) cells and NK T-cells (NKT) [42, 45].

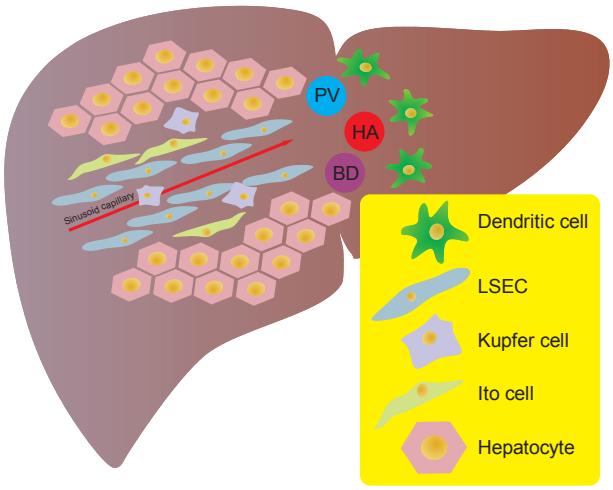

Figure 1 – ***Types of APCs typically found in the liver, and their distribution towards the hepatic sinusoids and portal spaces. LSEC: Liver sinusoid endothelial cell, PV: Portal vein, HA: Hepatic artery, BD: Bile duct. Kupffer cells extravasate between LSECs and interact with them. Ito cells can be found near sinusoid capillaries. Dendritic cells reside in the portal spaces.***

In the healthy liver, DCs are predominantly immature cells [44]. The majority of liver DCs can be found in areas adjacent to the portal spaces [50]. Murine liver DCs subsets differ from human liver subsets by their surface and intracellular markers [44, 45].

For the identification and purification of murine and human DCs, various cell surface markers have been used. At least four distinct DCs subsets are found in mouse liver: conventional myeloid DCs (CD11c+CD8α-CD11b+ or CD11c+ PDCA-1-), pDCs (CD11cloB220+ Ly-6C+CD11b- or PDCA-1+), CD8α+ DCs (CD11c+ CD8α+CD11b-) and NK DCs (NK1.1+CD11c+) [51]. It has been observed that, compared with mouse spleen, the incidence of pDCs in the liver is higher than those of myeloid and CD8α+DCs [52]. Murine liver pDCs secrete large amounts of IFN-I in response to microbial stimulation [53] and are not highly effective APCs in T-cell activation [42]. Additionally, they produce more IL-10 and less IL-12p70 than splenic pDCs [43], and express high level of IL-27p28, an IL-12 family cytokine that both APCs and T-cells function [54]. NK DCs expressed both APC and cytotoxic properties when studied *in vitro*, also being able to produce IFN-γ [52]. Conventional myeloid DCs in liver express tolerogenic phenotypes. After TLR ligation, they produce less IL-12, but more IL-10 and IL-27 [43].

Rat hepatic DCs are commonly identified by using the integrin molecule OX62. Only two major CD populations have been identified in rat livers, their respective phenotypes being ED1+ED2-OX6+ and ED1-ED2-OX6+ [51, 55].

Although human liver and blood DCs were both equally immature, each DCs has distinct subset compositions. In humans' liver, myeloid DCs (CD11c+CD11b+ BDCA1+) represent the most prominent liver DC subset. Unlike blood DCs that upon TLR4 ligation secrete multiple proinflammatory cytokines, liver DCs produced substantial amounts of IL-10 and therefore exhibit tolerogenic properties [56]. Human liver pDCs express blood DC antigens, therefore they are either BDCA2+/BDCA4+ HLA–DR+CD11c-CD123hi or CD4+CD11c- [51]. Liver pDC have been described as playing a critical role for in the development of oral tolerance [57]. No CD8α+ DCs subsets similar to that found in mice has been identified in the human liver so far.

DCs: role and therapeutic implications in HCC

This currently HCC treatment is based on the *Barcelona-Clinic Liver Cancer* (BCLC allocation system that divides patients into five stages (0, A, B, C and D) according to different prognostic variables [4]. Resection and local ablation of small lesions and transplantation are potentially curative therapies for early-stage disease [3, 4]. Unlike other cancers, which in advanced stages may respond to adjuvant chemotherapy or radiation, for HCC is no curative treatment for intermediate- or advanced-stage tumors. Neither chemotherapy nor radiation therapy will reduce HCC mortality [58]. Under these conditions, HCC prognosis remains very poor, and treatment options are few [7].

Thus, finding novel therapies for HCC remains an urgent need. In these circumstances, immunotherapy has appeared as an attractive option for improving outcome for cancer patients in advanced stage. Additionally, immunotherapy vaccine strategies have shown immunogenicity and less toxicity than current chemotherapy [59]. Although immunotherapy is not recommended for the clinical management of HCC patients by current guidelines [4], several different immunotherapy vaccine strategies have been investigated in the last decade for this disease [9]. Tumor cells express low antigen levels and, therefore they can escape surveillance of the immune

system [60]. Even though many of the mechanisms used by tumors to escape from immune-mediated rejection are now known on a cellular and molecular level, advances in cancer immunotherapy vaccine have been slower than for other forms of immunotherapy [61, 62]. Unlike preventive vaccines, which aim to induce the expansion of pathogen specific T-cells and to establish immune memory, cancer therapeutic vaccines are not designed to prevent diseases but rather aim at raising a specific immune response against existing tumor cells [61, 63]. Although HCCs are phenotypically and genetically heterogeneous tumors [64], several studies have suggested that impaired function of the DCs may be an important factor in the escape of the tumor from the immune control in cancer patients [60]. Various researches have identified the DCs as one of the key elements in the immune regulation, studies focusing on their role in anti-tumor immunity induction and, subsequently in cancer therapy, including HCC [10, 11, 60, 62]. DCs are most powerful type of APCs that play a key role in primary immune responses, tolerance and maintenance of immune homeostasis, and also represent an important bridge between the innate and adaptive immune systems [12, 13]. DCs can significantly improve the cytotoxicity of the effectors, due to a large quantity of dendrites, and of many types of surface molecules and receptors, as well as secreted cytokines [25, 33, 40]. Because of their ability to activate both anti-tumor specific T-cells and innate immune effector components, DCs vaccines represent a promising adjuvant immunotherapeutic approach for patients with HCC. DC can be activated with cytokines and TLR agonists such as IFN-γ or lipopolysaccharide [59]. Different methods for antigen loading of DCs such as peptide pulsing [65], whole protein [66], and viral vector-mediated transduction [67] have been used in an attempt to optimize anti-tumor responses.

There is ample evidence to justify therapeutic DCs vaccines in HCC. Decreased function of peripheral blood DCs in patients with HHC hepatocellular carcinoma is well established [68, 69]. In their study, Chen *et al.* [70] found significantly lower numbers of CD83-positive DCs (mature and activated dendritic cells) in liver tissue of patients with HCC compared with liver cirrhosis patients. The total number of DCs in the peripheral blood of patients with HCC was reduced. Moreover, the group did not find CD83-positive DCs associated with HCC nodules. Direct evaluation of DCs from HCC patients has demonstrated a decrease in the frequency of monocytoid DCs in peripheral blood of HCC patients and more importantly a functional defect of these cells leading to impaired stimulation of allogeneic T-cells.

Cytokines secreted by DCs have been shown to hold an important regulatory role in immune response activation. Therefore, several studies analyzed the expression of different cytokines. IL-12 has been shown to play an anti-tumoral role *in vivo*. Ormandy *et al.* observed that decreasing IL-12 production by myeloid DCs might be a possible cause of impaired stimulation of allogeneic T-cells and IL-12 directed therapies could reverse this phenomenon [71]. IL-10 has potent immunosuppressive effects on APCs and effector T-cells, and has been shown to turn DCs into tolerogenic DCs. In tumors, local production of IL-10 has been associated with the induction of tolerance towards the tumor in addition to general immunosuppression. Additionally, local production of IL-10 can lead to the exclusion of APCs from the tumor mass [60]. The research of Beckebaum *et al.* strongly suggest that in HCC patients, increased systemic levels of IL-10 may directly account for the alterations in the frequency and maturity of circulating DCs subsets [72]. Madhav *et al.* described the immune response to a single injection of immature DCs in two healthy subjects. In contrast to prior findings using mature DCs, the injection of immature DCs to both subjects led to the specific inhibition of multiple peptide (MP)-specific CD8 T-cell effector function in freshly isolated T-cells and the appearance of MP-specific IL-10-producing cells [73].

Several rodent models have been used for defining DCs-based immunotherapy. Using an experimental small murine HCC model, Lee *et al.* have shown that DC pulsed with murine hepatoma cell-lysate can be applied to treat small HCC effectively *in vivo*. The small hepatocellular tumors, up to 3×3 mm in diameter, were eradicated entirely in more than half of the experimental mice after two courses of DC treatments. Histological aspects of small HCC treated by DCs clearly show heavy infiltration of lymphocytes around the tumor, which explains the effective treatment of DC for small HCC. This study also showed that efficacy of DCs-based immunotherapy decreases while tumors grow [74]. Another study demonstrated that the intra-tumoral injection of IL-12 encoding plasmid followed by intra-tumoral DC vaccination led to the suppression of HCC and metastases in mice [75].

Unfortunately, a limited number of immunotherapy trials for HCC have been conducted based on several strategies [76].

Nakamoto *et al.* [77] evaluated the bioactivity and beneficial effects of DC infusion in HCC tissues following trans-catheter hepatic arterial embolization (TAE). Their study showed that the patients treated with OK-432-stimulated DCs prolonged the recurrence-free survivals when compared with controls treated by TAE alone. Additionally, authors reported that combination therapy using TAE together with immature DC infusion is safe for patients with cirrhosis and HCC. Another study reported similar results [78]. Zhang *et al.* studied DCs populations found in human peripheral blood and cord blood, and evaluated their potential in clinical application. The authors demonstrated that cord blood DCs can induce effector cytotoxicity more efficiently compared to peripheral blood DCs. This occurs mainly because the coexistence of mature and immature cord blood DCs can be synergetic and this association can be more effective than single mature DCs. Specific antitumor activity improved consecutively to double pulsing with tumor antigen on both cord DCs and cytotoxic T-lymphocytes (CTLs). Therefore, human blood DCs and cord blood DCs have a potential application in the clinical therapy of hepatocellular carcinoma. For it is low cost, autogenous peripheral blood DC and lymphokine-activated killer (LAK) cells can be acquired from patients whom are in good condition, whilst cord blood DCs can be used in patients who are in worse condition [79]. In a phase II study, Palmer *et al.* investigated the

safety and efficacy of vaccination with mature autologous DCs pulsed with tumor lysate in advanced HCC patients. The DCs were loaded *ex vivo* with multiple antigens from lysates of a liver cancer cell line and matured using the appropriate cytokine cocktail prior to reinfusion. The authors demonstrated that the vaccine is safe for intravenous administration. A partial response or stable disease was measured in 28% of patients. In addition, no evidence for autoimmunity was registered [80]. Another recent study conducted by El Ansary *et al.* [81] evaluated the safety and efficacy of the autologous-pulsed DCs vaccine compared to supportive treatment in advanced HCC patients, not suitable for radical or loco regional therapies. Patients were divided into two groups, group I (n=15) who received vaccination with mature autologous DCs pulsed *ex vivo* with a liver tumor cell line lysate and control group (n=15) received supportive treatment. Venous blood were obtained from each patient to generate DCs, which were subsequently identified by CD80, CD83, CD86 and HLA-DR expressions using flow cytometry. The safety and efficacy of DCs vaccine was evaluated at three- and six-month follow-up by clinical, radiological, and laboratory assessment. The authors concluded that autologous DC vaccination in advanced HCC patients is safe and well tolerated. Additionally, both CD8(+) T-cells and serum IFN-γ were elevated after DCs vaccine. The feasibility, safety and immune activity of a multiple tumor-associated antigen (TAA)-pulsed DC vaccine were confirmed in a phase I/II clinical trial performed by Tada *et al.* [82] in five patients with advanced HCC and liver cirrhosis. However, clinical response was detected only in one patient. The DC vaccine was prepared by pulsing DCs with cytoplasmic transduction peptide-attached α-fetoprotein, glypican-3 and MAGE-1 recombinant fusion proteins and cultivating them in the presence of maturation cocktail.

Unfortunately, the number of human clinical trials testing DCs vaccination in HCC currently is quite small.

Conclusions

There are few therapeutic options for intermediate and advanced HCC currently. As a result, HCC prognosis remains very poor in these stages of disease. In these circumstances, DCs vaccination of HCC patients appears as a promising treatment option in HCC. Although DCs vaccines are currently used in various stages of clinical trials, no vaccine has been approved so far for HCC. Taken together, all the data demonstrates an association between specific cellular immune response and the clinical benefit of DCs vaccines, even in advanced HCC stage. Further investigations and further improvement of DCs vaccines will be required to achieve more effective therapeutic efficacy in HCC.

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Author contribution

All authors have contributed equally in preparing this manuscript and thus share first authorship.

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