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Immunobiology of Monocytes/Macrophages in Hepatocellular Carcinoma

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1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and characterized by progressive development, high postsurgical recurrence and extremely poor prognosis [1–3]. The dismal outcome has been attributed to the highly vascular nature of HCC, which increases the propensity to spread and invade into neighboring or distant sites [4, 5]. Therefore, it is considered an urgent task to identify key prognostic factors of HCC and to elucidate the mechanisms of disease progression.

HCC is usually present in inflamed fibrotic and/or cirrhotic liver with extensive leukocyte infiltration [6, 7]. Thus, the immune status at different tumor sites can largely influence the biological behavior of HCC [6, 8, 9]. Several recent studies have shown that high infiltration of intratumoral regulatory T cells is associated with reduced survival and increased invasiveness in HCC [10, 11]. These findings are in accordance with the general view that the tumor microenvironment induces tolerance [12–14]. However, there is substantial evidence that the inflammatory response associated with cancers can also promote HCC progression by stimulating angiogenesis and tissue remodeling [6, 15, 16]. These findings strongly indicate that, besides inducing immune tolerance, HCC may also reroute the pro-inflammatory immune response into a tumor-promoting direction.

Macrophages (Mφs) constitute a major component of the leukocyte infiltrate in tumors. These cells are derived from circulating monocytes, and, in response to environmental signals, they acquire special phenotypic characteristics with diverse functions [17–19]. Recent studies have found that tumor environments co-opt the normal development of Mφs to dynamically activate the recruited monocytes in different niches of HCC. The malignant cells can thereby avoid initiation of potentially dangerous Mφ functions and create favorable conditions for tumor progression [20, 21]. Notably, the density of activated monocytes in the peritumoral stroma is selectively associated with vascular invasion and poor prognosis in HCC patients, whereas the increased infiltration of suppressive Mφs in the cancer nests is only correlated with the reduced survival of patients [22]. Thus, immune functional data of activated monocytes/Mφs in distinct cancer environments are essential for understanding their roles and potential mechanisms in HCC immunopathogenesis.

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In this chapter, we will summarize the current knowledge about the tumor immune microenvironment of HCC and the role of monocytes/Mφs in HCC progression, paying particular attention to the tissue micro-localization and phenotype of these cells. Additionally, we will describe the poly-directional communications between monocytes/Mφs and other stroma cells, including cytotoxic T cells, regulatory T cells, TH17/TC17 cells as well as neutrophils, and how activated monocytes in HCC repurpose the inflammatory response away from antitumor immunity and toward tissue remodeling and pro-angiogenic pathways. Finally, possible implications for the design of novel monocyte/Mφ-based immunotherapeutic strategies will be discussed.

2. Immune microenvironments of human HCC

After several decades of neglect, tumor microenvironments are once again an area of active research interest in cancer [23–25]. The biology of cancer cannot be understood simply by underlining the significance of the malignant cells but instead must encompass the contributions of the cancer microenvironment to tumorigenesis and disease progression.

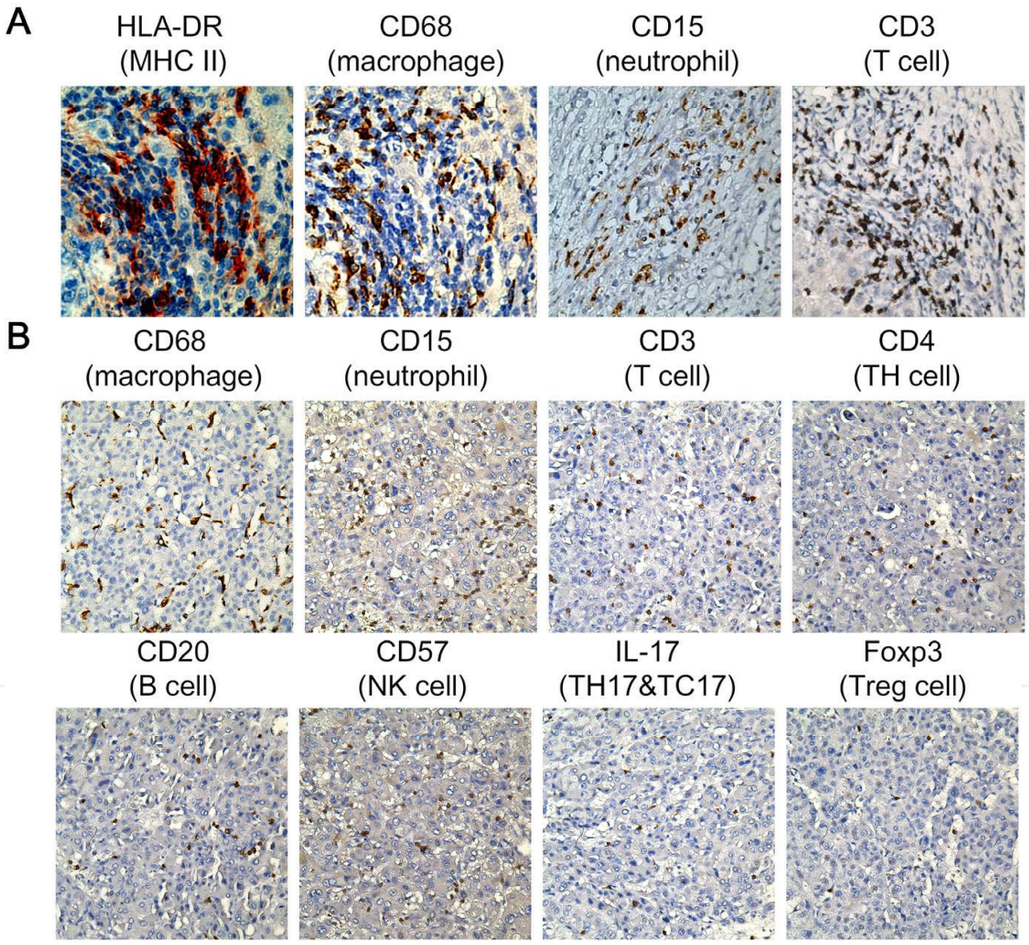

Fig. 1. **Infiltration patterns of immune cells in HCC samples.** Paraffin-embedded HCC sections were stained with indicated antibodies. The micrographs show the stained peritumoral stroma (A) and cancer nest (B).

Human HCC microenvironments, composed of non-cancer cells and their stroma (Figure 1), are now recognized as a major factor influencing the disease progression [6, 21]. Although normal stromal environment is non-permissive for HCC progression, hepatoma cells can modulate adjacent stroma to generate a supportive microenvironment. This includes the ability to alter the ratios of effectors to regulatory T cells and to affect the functions of APCs and the expression of co-signaling molecules, which in turn creates an immunosuppressive network to promote tumor progression and immune evasion [10, 21, 26]. However, there is also emerging evidence that the pro-inflammatory response at the tumor stroma can be rerouted in a tumor-promoting direction [15, 16]. These observations suggest that different tumor microenvironments can create either immune suppression or activation at distinct sites to promote tumor progression.

2.1 Immune responses against HCC

In HCC, several lines of evidence have suggest a positive role of immune system, e.g., by controlling hepatoma growth [7, 27–29]. Indeed, data from clinical investigations have revealed that HCC patients with an increased intratumoral accumulation of cytotoxic $CD8^+$ T lymphocytes had a superior 5-year survival rate and a prolonged recurrence-free survival after liver resection [11, 30]. In contrast, HCC-infiltrating $CD4^+$ T lymphocytes exhibited a $CD25^{high}Foxp3^+$ phenotype that was a predictor of poor overall survival of patients, indicating distinct roles of different tumor-infiltrating lymphocyte subsets in HCC [10, 11]. The important role of $CD8^+$ T cells in HCC control is further supported by studies in hepatoma-bearing mice; and interferon (IFN)-γ, perforin and granzyme B produced by $CD8^+$ T cells had been shown to be several major effector mechanisms for apoptosis of hepatoma cells [21, 31, 32].

Of note, the tumor-specific antigens (TSAs) in HCC patients are currently still under investigation. At present, several tumor-associated antigens (TAAs) of HCC have been identified, which has been recently introduced [7, 33]. In brief, several shared tumor antigens could also be recognized as antigens targeted by cytotoxic $CD8^+$ T cells in HCC, e.g., human telomerase-reverse transcriptase (hTERT) or NY-ESO-1. Expression of these antigens has been reported for other malignancies as well. Other antigens are expressed specifically in HCC and are also recognized by cells of the immune system, e.g., α-fetoprotein (AFP) or Glypican-3. The latter two antigens belong to the family of oncofetal antigens that are expressed only during ontogenesis. Although the exact mechanisms underlying are not yet clear, re-expression of such antigens is often observed in HCC. Thus, further research is also required to determine the frequency, immunodominance and strength of the immune responses induced against different TAAs.

Besides tumor-specific cytotoxic $CD8^+$ T cells, the natural killer (NK) cells have also been implicated in a successful immune response against HCC, e.g., by direct lysis of malignant cells [34]. Indeed, an increased preoperative NK cell activity that related to the expression of perforin and granzyme B was correlated with prolonged recurrence-free survival in HCC patients [35]. Contrarily, the NK cell dysfunction was shown to predict the poor survival of HCC patients after resection [36]. Other studies also showed that the stimulation of antigen-present cells (APCs) or natural killer T cells (NKT cells) can lead to an activation of NK cells and clearance of hepatoma cells in mice [37, 38].

Increased levels of B cells have been observed in several types of human tumor, and studies in mice indicate that, depending on microenvironment, tumor-infiltrating B cells are capable

of being pro- or anti-tumorigenic [39, 40]. The role of B cells in human HCC is unclear thus far. B cell-derived autoantibodies against several antigens have been described in mouse models of HCC as well as in HCC patients [41, 42]. Additional studies showed that monoclonal antibodies against Glypican-3 were able to induce antibody-dependent cellular cytotoxicity (ADCC) and thus lysis of human hepatoma cells in vitro and in mice [43]. However, the importance of ADCC and antibodies in general in the natural immune response against HCC has not been investigated so far.

2.2 Immune responses promote HCC progression

Although immune system can exhibit vigorous anti-hepatoma activities in vitro, HCC-specific immune responses fail to control tumor progression in most patients. Clinical and experimental studies have demonstrated that the growth of HCC is closely associated with impaired differentiation and maturation of APCs, particularly Mφs and dendritic cells (DCs) [20, 44, 45]. Also, phenotypic and functional analyses of APCs from HCC patients have revealed that tumor cells or tumor-derived factors do favor differentiation of monocytes into tumor-associated Mφs (TAMs) [20] or tolerogenic semi-mature DCs (TDCs) [46]. Also, such abnormal development of APCs in the HCC microenvironment could intensely impact the infiltration and function of other immune cells in tumor in situ, which will be expounded in the 3rd section of this chapter.

Suppression of immune responses by regulatory T (Treg) cells is one of the major mechanisms for the induction and maintenance of self-tolerance [12, 13]. Recent studies reported increased numbers of Treg cells in the peripheral blood and tumor-infiltrating lymphocytes (TILs) in patients with ovarian or liver cancers, which impaired cell-mediated immunity and promoted disease progression [10, 11, 47, 48]. Experimental depletion of Treg cells in several types of tumor-bearing mice could successfully improve tumor clearance and enhance the efficacy of immunotherapy [49, 50]. In parallel, depletion of $CD4^+CD25^+$ Treg cells could effectively enhance T-lymphocyte and NK-cell effector function in advanced stage HCC patients [51, 52]. These data together suggest that Treg cells may impair cell-mediated immune responses to HCC. At present, the precise underlying mechanism by which Treg cells accumulate at the tumor site in HCC patients is also unknown.

A direct role in HCC progression has also been shown for other cells of the immune system. For example, high infiltration of intratumoral neutrophils has been shown to predict a reduced recurrence-free survival time of HCC patients after liver resection [53]. Of note, neutrophils at the tumor edge induced angiogenesis progression and thus indirectly enhanced cancer growth [9]. This observation becomes especially intriguing in light of the finding that peritumoral neutrophils can be recruited by the pro-inflammatory cytokine interleukin (IL)-17. IL-17 is produced by T cells, termed TC17 or TH17, in the $CD8^+$ or $CD4^+$ T cell compartment in tumor environments, respectively [54, 55]. Indeed, IL-17-producing cells accumulate in tumors from patients with HCC and that their levels are positively correlated with microvessel density in tissues and poor survival in HCC patients [56]. It should be noted that, in several types of human cancer, high level of IL-17 in tumors in situ can predict improved survival of patients and associates with increased infiltration of cytotoxic $CD8^+$ T cells [57, 58]. Therefore, a better understanding of the network of tumor immune environments might provide a novel strategy for the rational design of anticancer therapies.

3. Polarization of monocytes in HCC microenvironments

APCs are critical for initiating and maintaining tumor-specific T-cell responses [21, 46]. DCs are considered the most effective APCs for primary immune responses [59]; Mφs markedly outnumber other APCs and represent an abundant population of APCs in solid tumors [60]. Monocytes can give rise to either DCs or Mφs in human tumors. In HCC patients, increased HLA-DR$^+$ monocytes in liver are associated with metastatic phenotype [6]. Thus, polarization of monocytes in the cancer environments is essential for understanding their roles in HCC immunopathogenesis.

3.1 Differentiation of monocyte-derived Mφs in HCC microenvironments

Mφs are essential components of host defense and act as both APCs and effector cells [19]. Under the influence of local conditions, they acquire specialized phenotypic characteristics with diverse functional programs [16–18]. Mφs constitute a major component of the leukocyte infiltrate of tumors, and the TAMs are derived almost entirely from circulating blood monocytes [17–19]. Mφs in normal or inflamed tissues exhibit spontaneous antitumor activity, whereas TAMs are polarized M2 cells that suppress antitumor immunity and promote tumor progression [61]. Those findings agree with clinical studies showing that a high density of TAM is associated with poor prognosis in most solid tumors [22, 62]. Although the precise underlying mechanisms are not yet clear, it is generally assumed that the tumor microenvironment is critical determinants of the phenotype of local Mφs. Tumor-derived factors, including IL-10 and transforming growth factor (TGF)-β, "educate" the newly recruited monocytes to take on a M2 phenotype and perform a protumoral role [63, 64]. In contrast, over-expression or local delivery of IL-12 can reestablish the antitumor activity of Mφs, and in that case a high density of TAMs is correlated with a marked reduction in tumor growth [65, 66]. Such opposing effects of Mφs on tumor progression indicate that selective modulation of Mφ polarization might serve as a novel strategy for cancer therapy. However, such an approach is hampered by the fact that the mechanisms by which tumor microenvironments educate Mφs to perform specific tasks have not been fully elucidated.

In HCC patients, soluble factors derived from hepatoma cells, including extracellular matrix components hyaluronan fragments [20], effectively induced the formation of TAMs. Interestingly, kinetic analysis revealed 2 opposing functional stages in the TAM life cycle: monocytes are rapidly activated during a narrow time window, 4 to 16 hours after their first exposure to hepatoma cell culture supernatants, and afterward the same cells become exhausted and their production of cytokines is extinguished, with the exception of IL-10 [20, 21]. Because TAMs are derived from blood monocytes, such sequential pre-activation and exhaustion of cells may reflect a novel immune-escape mechanism by which tumors dynamically regulate the functions of migrating monocytes at distinct tumoral sites. More precisely, this means that during their first exposure to the tumor microenvironment, the newly recruited monocytes may be transiently activated while they are approaching the stroma surrounding the tumor, with the aim of minimizing their potential to damage tumor cells. Thereafter, when these Mφs are in close proximity to the tumor cells, they become exhausted and thus fail to mount an effective antitumor immune response. This notion is supported by the observations in human HCC tissues, indicating that most CD68-positive cells are smaller and show high expression of HLA-DR in the peritumoral stromal region, whereas they exhibit a HLA-DR^{low}IL-10^{high} phenotype in the cancer nest (Figure 3 in Ref. 20).

Of note, similar activation patterns of monocytes/Mφs were detected in ovarian and lung cancer [20, 67].

Monocytes/Mφs in the peritumoral stroma of HCC had an activated phenotype with increased expression of HLA-DR, CD80, and CD86 [6]. Data from in vivo observations showed that such tumor-activated monocytes also expressed significant level of B7-H1 (PD-L1) [8, 21]; and autocrine TNF-α and IL-10, but not IFN-γ, released from activated monocytes, stimulated monocyte B7-H1 expression [21, 68]. Furthermore, in vitro study using recombinant TNF-α and IL-10 indicated that IL-10 was essential for B7-H1 induction and that pro-inflammatory TNF-α acted synergistically with anti-inflammatory IL-10 to enhance B7-H1 expression on monocytes. In addition, a positive correlation between IL-17-producing cells and B7-H1-expressing Mφs was also observed in the peritumoral stroma of HCC tissues (Figure 1 in Ref. 8). Although culture supernatants derived from hepatoma cells also induced B7-H1 expression on monocytes, IL-17 additionally increased hepatoma-mediated monocyte B7-H1 expression. Similar regulatory effect of IL-17 on APCs was also observed in hepatitis [69]. These findings reveal a fine-tuned collaborative action between different types of immune cells in the peritumoral stroma of HCC, which reroutes the monocyte inflammatory response into immunosuppression.

TAMs in the cancer nests of HCC exhibited an exhausted suppressive phenotype; they are strongly impaired with regard to various functions related to inflammation [20, 21]. However, in contact with autologous T cells, these suppressive Mφs recuperate their capabilities to produce low level of IL-12 in tumors, and thereby activate T cells to produce IFN-γ, which in turn leads to IDO expression in Mφs and ultimately impairs the antitumor T cell immunity (Zhao Q, et al. J Immunol. doi: 10.4049/jimmunol.1100164). These findings give important new insights into the collaborative action of tumor stroma cells that is exercised to counteract the normal development of Mφs in distinct HCC environments.

3.2 Differentiation of monocyte-derived DCs in HCC microenvironments

DCs are the most potent "professional" APCs, and they are responsible for integrating a variety of incoming signals and orchestrating the immune response [59]. Bidirectional interactions between DCs and T cells initiate either an immunogenic or a tolerogenic pathway, both of which play crucial roles in autoimmune diseases and tumor immunity [70, 71]. It is generally assumed that these two contrasting functions of DCs are associated with the maturation stages of the cells: fully mature DCs (mDCs) are efficient activators of naive T cells, whereas immature DCs (iDCs) have been implicated for anergy induction. Furthermore, an intermediate stage of maturation was recently described in which the cells are referred to as semi-mature DCs [72]. These DCs expressed high levels of MHC class II and co-stimulatory molecules, even though they exhibited an IL-12^{low}IL-10^{high} phenotype [73]. It was also observed that the semi-mature DCs can induce tolerance by generating regulatory T cells and/or T cell anergy [74].

In HCC patients, soluble factors derived from hepatoma cells drove human monocytes to become tolerogenic DCs (TDCs) that exhibited a semi-mature phenotype with a 2- to 5-fold increase in expression of CD83, CD86, and HLA-DR, and a distinctive IL-12^{low}IL-10^{high} cytokine production profile [46]. Upon encountering T cells, the TDCs triggered rapid down-regulation of CD3ε and TCR-α/β and subsequent apoptosis in autologous T cells.

Consistent with these results, accumulation of immunosuppressive DCs coincided with CD3ε down-regulation and T cell deletion in the cancer nests of human HCC tumors (Figure 2 and 4 in Ref. 46). The impaired T cell function was mediated by factor(s) released by live TDCs after direct interaction with lymphocytes. Also, the TDC-induced effect on T cells was markedly reduced by blocking of NADPH oxidase but not by inhibition of arginase, inducible NO synthase (iNOS), indoleamine 2, 3-dioxygenase (IDO), or IFN-γ. These observations indicate that tumor microenvironments educate DCs to adopt a semi-mature phenotype, which in turn aids tumor immune escape by causing defects in the CD3/TCR complex and deletion of T cells. In addition, besides triggering T cell apoptosis, hepatoma-exposed DCs also play an important role in expanding intratumoral Treg cells [45].

4. Cross talks between monocytes/Mφs and other stroma cells in HCC tissues

Tumor progression is now recognized as the product of evolving crosstalk between different cell types within the tumor and its stroma [23, 24]. HCC environments can alter the normal development of Mφs that is intended to trigger transient early activation of monocytes in the peritumoral region, which in turn induces formation of suppressive TAMs in the cancer nests [20, 21]. In this section, we will describe several recent findings about the cross talks between monocytes/Mφs and other stroma cells, paying particular attention to the tissue micro-localization and phenotype of these cells in HCC.

4.1 Activated monocytes in the peritumoral stroma of HCC foster immune privilege and disease progression through B7-H1

B7-H1 is a cell-surface glycoprotein belonging to the B7 family of co-signaling molecules with a profound regulatory effect on T cell responses [75]. Studies in mouse models have revealed that expression of B7-H1 helped dormant tumor cells to evade cytotoxic T cell responses [14]. In human HCC, B7-H1$^+$ monocytes/Mφs and CD8$^+$ T cells were both accumulated in the peritumoral stroma of HCC tissues, which suggests that these monocytes/Mφs promote tumor progression by impairing T cell immunity (Figure 1, 3-5 in Ref. 21). Supporting this hypothesis, a significantly larger portion of the tumor-infiltrating cytotoxic T cells was found to express the B7-H1 receptor PD-1 [8, 21, 68]. Moreover, HCC-infiltrating T cells co-cultured with HCC-derived monocytes exhibited an impaired production of IFN-γ, and blockade of B7-H1 by pre-incubation of tumor monocytes with the mAb MIH1 could markedly enhance the ability of tumor T cells to produce that cytokine. Consistent with these data, only PD-1$^+$ cytotoxic T cells isolated from HCC tissues exhibited attenuated production of IFN-γ, IL-2, and TNF-a as well as low cytotoxic potential [21]. These findings, together with regulatory mechanism of B7-H1 in monocytes/Mφs (Section 2A), suggest that there is a fine-turned collaborative action between immune activation and immunosuppression in tumor microenvironments. Soluble factors derived from hepatoma cells [21], as well as IL-17 released by TH17 and TC17 [54, 55], can trigger transient activation of newly recruited monocytes in the peritumoral stroma area of HCC, and thereby induce the monocytes to produce significant amount of cytokines, including TNF-α, IL-23, IL-1β, and IL-10, which in turn leads to the expression of B7-H1 protein on their surface and ultimately impairs the antitumor T cell immunity.

4.2 Activated monocytes in the peritumoral stroma of HCC promote expansion of memory IL-17-producing T cells

Although cancer patients exhibit a generalized immunosuppressive status, substantial evidence indicates that the inflammatory reaction at a tumor site can promote tumor growth and progression. HCC is usually derived from inflamed cirrhotic liver with extensive leukocyte infiltration. Recent study has shown that TH17 cells were highly enriched in HCC and their levels were positively correlated with micro-vessel density in tissues and poor survival in HCC patients [56]. In contrast to the classical TH17 cells that hardly express IFN-γ, almost half of the IL-17-producing $CD4^+$ T cells we isolated from HCC tissues were able to simultaneously produce IFN-γ, suggesting that the tumor microenvironment can profoundly determine the phenotype of such cells [54–56]. In addition, the IL-17-producing cells often predominantly accumulate in the peritumoral stroma rather than in the cancer nests of HCC [54, 55]. A significant correlation between the levels of $CD68^+$ cells and $IL\text{-}17^+$ lymphocytes was found in the peritumoral stroma of HCC (Figure 1 in Ref. 54). However, there was no such correlation in intratumoral tissue, suggesting that Mφs in different parts of HCC play disparate roles in IL-17-producing T cell expansion.

Most of the $CD68^+$ cells in the peritumoral stroma had a smaller volume and showed marked expression of HLA-DR, which implies that they were newly recruited and activated monocytes. In contrast, most Mφs in the cancer nests were negative for HLA-DR (Figure 2). Accordingly, hepatoma-activated monocytes were significantly superior to the suppressive tumor Mφs in inducing expansion of both TH17 ($CD4^+$ IL-17-producing T cells) and TC17 ($CD8^+$ IL-17-producing T cells) cells from circulating memory T cells in vitro with phenotypic features similar to those isolated from HCC, and these monocytes secreted a set of key pro-inflammatory cytokines, including IL-1β, IL-6, and IL-23, that triggered proliferation of functional TH17 cells. In addition, inhibition of monocytes/Mφs inflammation in liver markedly reduced the level of tumor-infiltrating $IL\text{-}17^+$ cells and tumor growth in vivo [54]. Therefore, the pro-inflammatory IL-17-producing cells are

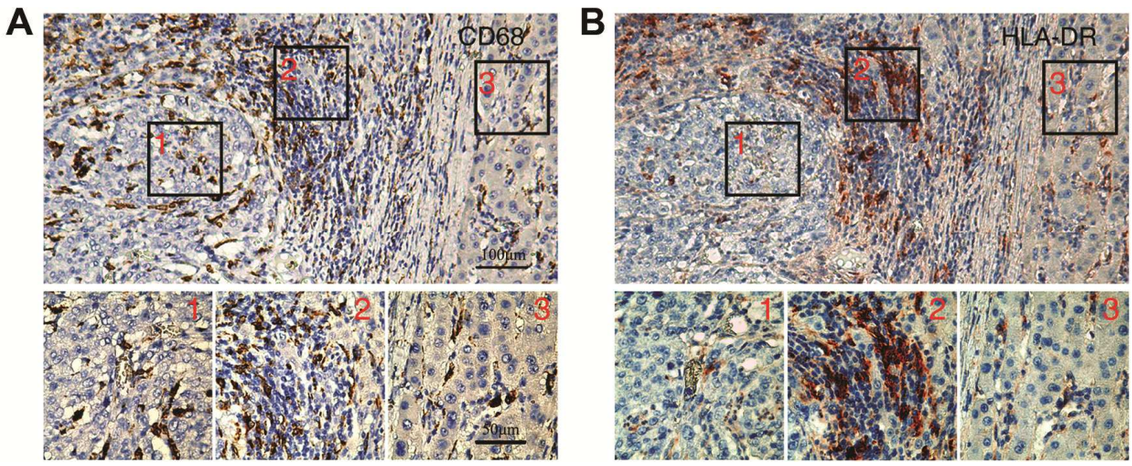

Fig. 2. **Distinct activation patterns of monocytes/Mφs in HCC samples.** Adjacent sections of paraffin-embedded HCC samples were stained with an anti-CD68 (A) or anti-HLA-DR (B). The micrographs at higher magnification show the stained cancer nest (1), peritumoral stroma (2), and adjacent normal tissue (3).

generated and regulated by a fine-tuned collaborative action between different types of immune cells in distinct HCC microenvironments, and allow the inflammatory response of activated monocytes to be rerouted in a tumor-promoting direction. Selectively modulating the "context" of inflammatory response in tumors might provide a novel strategy for anticancer therapy.

4.3 Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in HCC

Most monocytes/Mφs in the peritumoral stroma of HCC exhibit an activated phenotype that favors the generation of IL-17-producing cells in the same area, and their levels were correlated with disease progression in HCC patients [54]. Interestingly, another important myeloid cell population, namely tumor-associated neutrophils (TANs), was also enriched predominantly in the peritumoral stroma of HCC tissues, and their levels were well correlated with IL-17-producing cell density in the same area (Figure 2 in Ref. 9). Data from both clinical sample analysis and experimental studies showed that functional IL-17$^+$ cells in the peritumoral stroma stimulated epithelial cells to produce CXC chemokines that induced neutrophil trafficking to tumors [9]. Thereafter, exposure of neutrophils to HCC environment resulted in sustained survival of cells [76]. The accumulated neutrophils in the peritumoral stroma were the major source of MMP-9, which in turn triggered the angiogenic switch at the adjacent invading edge [9]. These data, therefore, provide direct evidence that neutrophils play an important role in human tumor progression by serving as a link between the pro-inflammatory response and angiogenesis in the tumor milieu. This notion is supported by the findings that the density of neutrophils in the peritumoral stroma was correlated with advanced disease stages and could serve as an independent predictor of poor survival in HCC patients (Figure 1 in Ref. 9). Consistent with these results, tumor angiogenesis is often more active at the invading edge, which is close to the peritumoral stroma, than intratumoral areas.

4.4 Activated CD69$^+$ T cells foster immune privilege by regulating Mφ IDO expression in the cancer nest of HCC

IDO is a rate-limiting enzyme for tryptophan catabolism. In humans and mice, IDO inhibits antigen-specific T cell proliferation in vitro and suppresses T cell responses to fetal alloantigens during murine pregnancy [71, 77, 78]. Expression of IDO is often induced or maintained by many inflammatory cytokines, of which IFN-γ is the most potent [71]. In addition to being expressed in APCs, most human cancers also express high levels of IDO protein which correlates with poor prognosis in some cases [78]. In contrast, low or rare IDO expression is observed in most mouse and human tumor-cell lines, possibly due to the lack of a complete cancer microenvironment in cell lines in vitro [78, 79]. At present, little is known about the regulating mechanisms of IDO in TAMs at stroma of human tumors in situ.

Experimental studies indicate that the IDO proteins are selectively high expressed by Mφ in several types of human tumors, including HCC (Zhao Q, et al. J Immunol. doi: 10.4049/jimmunol.1100164). However, exposure to hepatoma cell culture supernatants did not elicit the IDO expression in monocytes/Mφs, which suggests that additional factors within the tumor milieu are required for inducing IDO expression in tumor Mφs. CD69 is an

immunoregulatory molecule expressed by early activated leukocytes at sites of chronic inflammation and $CD69^+$ T cells have been found to promote human tumor progression [80, 81]. Upon encountering autologous $CD69^+$ T cells, tumor Mφs acquired capabilities to produce greatly higher amount of IDO protein. The T cells isolated from the HCC tissues expressed significant CD69 molecules than those on paired circulating and non-tumor-infiltrating T cells; and these tumor-derived $CD69^+$ T cells could induce considerable IDO in monocytes. Interestingly, the tumor-associated monocytes/Mφ isolated from HCC tissues or generated by in vitro culture effectively activated circulating T cells to express CD69. IL-12 derived from tumor Mφ was required for early T cell activation and subsequent IDO expression (Zhao Q, et al. J Immunol. doi: 10.4049/jimmunol.1100164). Consistent with this, another recent study has shown that intratumoral delivery of exogenous IL-12 could elicit an IFN-γ-dependent IDO counter-regulation in mouse model [82]. Moreover, the conditioned medium form IDO^+ Mφ effectively suppressed T cell responses in vitro; an effect which could be reversed by adding extrinsic IDO substrate tryptophan or by pre-treating Mφs with an IDO-specific inhibitor 1-MT. Such an active induction of immune-tolerance should be considered for the rational design of effective immune-based anti-cancer therapies.

4.5 Increased intratumoral Treg cells are related to intratumoral Mφs and poor prognosis in HCC patients

Treg cell-mediated immunosuppression is a crucial strategy of tumor immune evasion and a main obstacle for successful cancer immunotherapy [12]. Several recent studies have demonstrated that $FoxP3^+$ Treg cells with immunosuppressive properties were concentrated within HCC tumors and that the intratumoral prevalence of $FoxP3^+$ Treg cells was associated with disease progression and poor prognosis [10, 11]. However, the source of Treg cells in HCC is still unclear. By analyzing the association between the densities of Treg cells and Mφs in HCC tissue, Zhou et al observed that the elevated intratumoral $FoxP3^+$ Treg population was correlated with high-intratumoral Mφ density in HCC patients [26]. Depletion of liver Mφs thus decreased the frequency of liver $FoxP3^+$ Treg cells in hepatoma-bearing mice. Additionally, Mφs exposed to TSNs from hepatoma-derived cell lines augmented the $FoxP3^+$ Treg population, partially via IL-10. However, in the absence of Mφs, culture supernatants derived from hepatoma cells could not increase the percentage of $FoxP3^+$ Treg cells upon anti-CD3/CD28 stimulation. These data indicated that HCC-associated immunosuppressive Mφs can increase the intratumoral $FoxP3^+$ Treg population.

Cross talks between monocytes/Mφs and other stroma cells are summarized in Figure 3. Notably, the regulatory mechanisms of monocytes/Mφs are less well understood in human HCC. Besides interacting with TH17, TC17, Treg, and $CD69^+$ T cells, tumor-associated monocytes/Mφs also secrete molecules (eg, TGF-β, osteopontin), which directly induced epithelial-mesenchymal transition or transformation (EMT) of cancer cells [83]. Moreover, one of our latest finding indicated that monocytes/Mφs isolated from human HCC tissues induced NK cell dysfunction via a 2B4/CD48 interaction (Wu et al. Unpublished data). In addition, although not directly related to HCC, tumor-infiltrating Treg cells can trigger production of IL-10 by Mφs, which in turn stimulates such cells to express B7-H4 in an autocrine manner and renders them immunosuppressive via the B7-H4 molecules [84]. Therefore, manipulating the involved molecules may open new avenues for developing novel immune-based therapies to enhance antitumor immunity in human cancer.

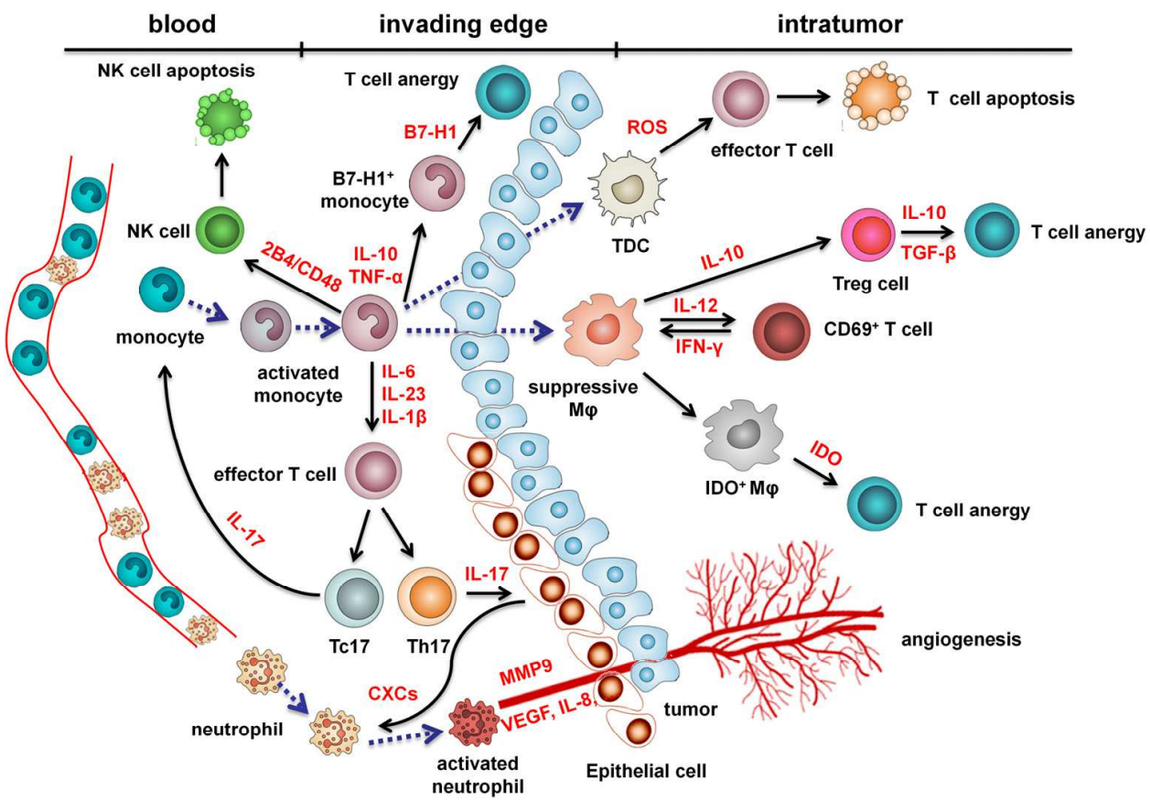

Fig. 3. Map of Cross talks between monocytes/Mφs and other immune cells in distinct niches of HCC.

5. Summary

Much research has been focused on tumor-mediated immunosuppression over the past decade. However, in spite of the generalized immunosuppressive status in cancer patients, many malignancies arise at sites of chronic inflammation, and inflammatory mediators are often produced in tumors [85, 86]. Human HCC tissues can be anatomically classified into areas of intratumoral and peritumoral stroma, each with distinct compositions and functional properties [20, 21, 54, 55]. Intratumoral environments of HCC usually contain abundant immunosuppressive molecules and cells to inhibit the T cell responses and create conditions that are conducive to tumor growth [20, 26]. In contrast, the peritumoral stromal areas of HCC contain a significant amount of leukocyte infiltrate, which are thereby situated close to the advancing edge of a tumor [21, 54]. Monocytes/Mφs represent an abundant population of APCs in HCC. Soluble factors derived from hepatoma cells can alter the normal developmental process of Mφs that is intended to dynamically regulate monocyte activation at distinct sites [20]. Hepatoma-activated monocytes in the peritumoral stroma induce sequential expansion of memory TH17 and TC17 cells and infiltration of neutrophils to promote inflammation and angiogenesis at invading tumor edge [54, 55]. Of note, these activated monocytes also express high level of B7-H1 to inhibit tumor-specific T cell immunity [21], and in that way repurpose the inflammatory response away from anti-tumor immunity (the sword) and towards tissue remodeling and pro-angiogenic pathways (a

plowshare). Furthermore, interactions between suppressive Mφs and T cells in the cancer nest of HCC lead to Mφ IDO expression and Treg cell expansion [26]. Thus, it is not inflammation per se but inflammatory "context" that determines the ability of pro-inflammatory factors to facilitate or prevent tumor growth. Studying the mechanisms that can selectively modulate the functional activities of monocytes/Mφs might provide a novel strategy for anticancer therapy.

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Tumor microenvironment represents an extremely dynamic niche shaped by the interplay of different cell types (e.g. tumor cells, stromal cells), their soluble products (e.g.cytokines, chemokines and growth factors) and varied physico-chemical conditions (e.g low oxygen concentration or hypoxia). Recent studies have identified myelomonocytic cells as key players in regulating the tumor microenvironment and hence, tumor progression in a variety of cancers. In view of these findings, the present book attemps to provide a comprehensive account of the diversity of tumor microenvironment across different cancers and how myelomonocytic cells have taken the center-stage in regulating this niche to direct cancer progression. A better understanding of the myelomonocytic cells and the mechanisms by which they regulate cancer progression will open new vistas in cancer therapeutics.

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