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**IMMUNOLOGY OF HEPATOCELLULAR CARCINOMA**

**Running title** Immunology of HCC

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**Abstract**

Hepatocellular carcinoma (HCC), is primarily a malignancy of liver, advancing from a damaged, cirrhotic liver to HCC. Globally, HCC is the sixth most prevalent cancer and the third-most reason of neoplastic disease-related deaths. A diverse array of infiltrating immunocytes just like in many other cancers also regulates the development and progression of HCC. An understanding of various immune components during HCC becomes necessary so that novel therapeutic strategies can be designed to combat the disease. A dysregulated immune system, including changes in the number and/or function of immune cells, cytokine levels and expression of inhibitory receptors or their ligands, plays a key role in the development of HCC. Alterations in either innate or adaptive arm of the immune system and a cross-talk between them make the immune system tolerant to tumor, leading to progression of the disease. In this review, we have discussed the status and role of various immune effector cells, e.g. dendritic cells, natural killer cells, macrophages, T cells, their cytokine profile and the chemokine-receptor axis in promoting or impeding the hepatocellular carcinoma.

**Key words:** Hepatocellular carcinoma, immune cells, innate immunity, adaptive immunity, immune-dysregulation

**Core tip:** Hepatocellular carcinoma is a heterogeneous disease caused by multiple factors, and its immunopathogenesis is complicated by the paradoxical role of various immune cells. This review provides a comprehensive insight into the immunological mechanisms that control hepatocarcinogenesis. A better and fuller understanding of the precise functioning of each of the cellular subsets may open new avenues for the treatment of HCC.

**Introduction**

Hepatocellular carcinoma (HCC) is a cancer that emanates in the liver and is different from the metastatic liver cancer that hails from some other organ and culminates in the liver. Worldwide, HCC stands as the sixth most prevalent cancer, a third most cause of mortality and a common poor-prognosis malignancy due to recurrence after surgery and metastasis[1]. It accounts for approximately 70-80% of all primary liver cancer cases[2]. It is most prevalent in Asian nations like China and Japan, and a leading cause of death within few weeks or months of detection. The disease is generally diagnosed at a late stage, which significantly brings down the survival rate to less than 14% within a span of five years[3]. The available treatment options are not 100% successful and the estimated recurrence rates are around 50% over a span of 3 years post-surgery and with a survival rate of only 30-40% at five years post-surgery[4].

The major risk factors for chronic liver disease and subsequent HCC include prior infection with viruses like hepatitis B and hepatitis C[5]. Studies in mouse models have pointed towards a major role of local intra-hepatic chronic inflammation in promoting hepatocarcinogenesis in animals with non-alcoholic steatohepatitis (NASH)[6]. Accumulating data in humans also indicate an increasing role for NASH as a risk factor for HCC development [7]. Besides these, other emerging risk factors are obesity, especially visceral adiposity leading to non-alcoholic fatty liver disease (NAFLD), consumption of alcohol, tobacco, foodstuffs contaminated with aflatoxin B1, diabetes, over use of oral contraceptive pills, and iron overload[4].

The factors promoting tolerance to tumor antigens, including decreased recognition of malignant cells and suppression of immunity, chronic inflammation either mediated by virus[8] or immune dysregulation, all lead to carcinogenesis[9]. Recent studies have provided evidence that dysregulated immune system, including changes in the number or function of immune cells, cytokine levels and expression of inhibitory receptors or their ligands significantly contribute to the development of HCC [10, 11]. Alterations in the functioning or expression of immune components shift the immune response towards tolerance to tumor resulting in its progression. Tumor-related immune cells, such as cytotoxic T cells, CD4+ T cells, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells and a cross-talk between these have all been reported to be involved in the development of HCC (**Figure 1**). In this review, we have discussed the immunology of HCC in terms of status of various immune effector cells.



**Figure 1: Role of immune cells in HCC.** As the disease progresses from cirrhosis of liver to HCC, the functions of various immune cells gets dysregulated. The DCs lose their antigen presentation capabilities with reduced secretion of Th1 cytokines. The macrophages differentiate into an 'alternatively activated phenotype' that generates a Th2-type of immune response promoting Tregs recruitment and development. The NK cells have reduced cytolytic activities. The T cells, both CD4+ and CD8+ T cells decrease in numbers with attenuated function and increased expression of inhibitory receptors during HCC. Th17 cells increase in numbers and correlate with angiogenesis and poor-prognosis. The Tregs exert negative effects on T cells, DCs and NK cells and may promote the differentiation of Th17 cells via the immunosuppressive cytokines. There is shift in overall cytokine *milieu* from a Th1 to Th2 profile.

DCs-dendritic cells; NK-natural killer cells; Tregs-regulatory T cells

**Innate immune system**

*Dendritic cells*

An efficient recognition, processing and presentation of tumor antigens by the dendritic cells (DCs) is a prerequisite for an effective immune response against tumors. Overall, a failed HCC-associated antigen presentation by DCs might not be only due to a decreased expression of HLA class-I molecules [11], but also the maturation defects like reduced endocytosis, allostimulation and IL-12 secretion leading to a weak T cell immune response [12]. Even in the presence of strong maturation stimuli like high levels of inflammatory cytokines, DCs remain refractory to these stimulatory signals. It has previously been shown in studies that there is a numerical and functional defect in the peripheral DCs in HCC patients with hepatitis B and C virus infections, although it is not clear whether this defect in DCs is a cause or effect [13, 14]. Reports, on the other hand, have shown that the frequency of activated CD83+ DCs in the peripheral circulation of HCC patients was comparable to patients with liver cirrhosis and normal healthy controls [15]. However, as compared to peripheral blood, activated DCs were present at a much lower frequency than the other study groups in liver tissues. Additionally, the activated DCs in HCC patients were not able to infiltrate the cancer nodules resulting in impaired recruitment of tumor-specific lymphocytes to tumor areas.

Recently, a new regulatory subset of DCs called CD14+CTLA-4+ DCs expressing inhibitory molecules like cytotoxic T-lymphocyte-associated protein (CTLA)-4 and programmed death receptor (PD)-1 were observed in peripheral blood lymphocytes and tumor mass of HCC patients[16]. High levels of anti-inflammatory cytokine, IL-10 and Indoleamine 2, 3-dioxygenase (IDO) secreted by these cells' post-stimulation suppressed the CD4+ T-cell immune responses helping tumor immune escape and progression of tumors.

*Macrophages and myeloid derived suppressor cells*

 Tumor-associated macrophages (TAMs) represent the main inflammatory cells associated with cancer-related inflammation[17]. While infiltrating tumors, TAMs differentiate towards an M2 phenotype which is characterized by poor antigen presentation capacity and expression of immunomodulatory cytokines, like IL-10 and TGF-β. TAMs also express chemokines like CCL17, CCL22 and CCL24, along with arginase but low levels of proinflammatory cytokines and reactive oxygen species[18]. In HCC, the cytokines, IL-6 and TGF-𝛽, in particular, favour tumor growth, others like TNF-𝛼 with IL-6 are involved in invasion and metastasis whereas TGF-𝛽, in concert with IL-10 has been shown to promote the suppression of antitumor immune response[19]. This alternative phenotype of macrophages further participate in the activation of a T helper type 2 (Th2) immune response promoting the recruitment and development of regulatory T cells (Tregs). The chronic inflammation was reported primarily to be coupled with a higher prevailing level of macrophage colony stimulating factor and a higher infiltration of macrophages, which were reportedly associated with HCC progression and intrahepatic metastasis, signifying the role of TAMs in the recurrence and metastasis of HCC[20, 21].

Another heterogeneous population of cells, called myeloid-derived suppressive cells (MDSCs), which are a subset of inflammatory monocytes have been identified that comprise of immature myeloid progenitors that are not already committed into any cell lineage [20]. They can exert inhibitory functions, and regulate T cell responses through the upregulated expression of several factors like free radicals, arginase activity and production of TGF-β, encouraging the induction of Treg cells[22]. Like typical monocytes, these cells express CD14 but have a lower or no expression of HLA-DR. An increased frequency of these cells has been reported in the peripheral circulation and tumor environment of HCC patients[23].

Similarly, neutrophils being a common inflammatory infiltrate in tumors, could also provide a prediction of poor survival in HCC patients since their numbers correlated positively with the stage of cancer. Kuang *et al*[24] demonstrated that the peritumoral stromal cells were fortified with neutrophil populations under the influence of Th17 cells through the chemokines like CXCL8 produced by epithelial cells. These neutrophils produce proteases like matrix metalloproteinase-9 in HCC tissues, promoting angiogenesis. Thus, neutrophils provide a connection between the immune cells and angiogenesis and promote tumor growth.

*Natural Killer cells*

An exaggerated cytolytic population of NK cells serves as immune invigilators in the liver microenvironment[25]. NK cells are cytotoxic and regulate the activity of other immune cells through the cytokines they release[26]. Under normal physiological conditions, the NK cells mediate their functions in liver via the production of 'cytolytic granules' containing perforin, granzymes, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and IFN-γ [27]. However, their functions are not completely imparted in case of many cancers, including HCC. For instance, in HCC patients, a significant decrease in the CD56dim NK subsets in the peripheral blood has been reported as compared to healthy subjects[28]. A significantly lower proportion of these NK cell subsets exhibiting reduced levels of IFN-γ and cytotoxic potential has also been reported in tumor regions compared with non-tumor regions in HCC patients[29]. Multiple mechanisms have been put forward to explicate the decreased functioning of NK cells and their association with cirrhosis of the liver and cancer, including fibrotic damage to NK cells[30], phagocytic uptake of NK cells by activated hepatic stellate cells[31], and the upregulation of inhibitory or down-regulation of activating receptors respectively[32].

**Adaptive Immune system**

*T lymphocytes*

T lymphocytes, both CD4+ T helper cells and CD8+ cytotoxic T cells are mostly considered to be significant players in inhibiting, impeding and killing tumor cells. Their existence in the cancer areas has been observed and correlated with a favourable prognosis in many cancers[33]. The IFN-γ produced during the Th1 immune response play a crucial role in the evasion and amelioration of HCC. In addition to helper T cells, the role of cytotoxic CD8+ T cells during HCC disease have been evaluated in many studies, and a significant role is attributed to them in the killing of tumor cells.

In one study, it was reported that there was a significant decrease in [CD4+ T-cells](http://hinarilogin.research4life.org/search?dc.title=CD4+T-cells&facet-content-type=ReferenceWorkEntry&sortOrder=relevance) in patients with liver cirrhosis and HCC indicating their importance in facilitating malignancy among the cirrhotic patients[34]. They have, likewise, noticed a decreased ratio of helper T cells/suppressive T cells in peripheral blood of patients with liver cirrhosis and HCC. Upon assessment of the genetic profile, a gene signature consisting of 17 immune related genes, changing the tumor microenvironment from a Th1 to a Th2 type *milieu* has been identified [20]. This foreshadows the development of venous metastasis in HCC and impaired disease outcome indicating that progression of liver diseases is linked with a dysregulated cellular immune response.

Several mechanisms to deduce the immunosuppressive nature of T cells have been explained by many authors. Previous studies have shown increased levels of the soluble IL-2 receptor alpha chain, CD25 in the serum of cancer patients[35] [36]. These studies have shown positive correlation between sCD25 and disease severity serving as a surrogate indicator of survival and response to therapy[35]. The serum of HCC patients was evaluated and revealed elevated levels of sCD25 as compared to the normal, healthy individuals and patients with cirrhotic livers [36]. The authors observed an improvement in T cell responses after sCD25 depletion suggesting that sCD25 has indeed been involved in suppressing effector T cell functions.

Many human cancer cells express the ligand for inhibitory receptor, PD-1. An upregulated expression of its ligand, PD-L1 on intra-tumoral kupffer cells and concurrent increase in PD-1 expression on CD8+ T cells is detrimental in cancer[37]. Moreover, the MDSCs were also found to have upregulated expression of PD-L1 leading to functional exhaustion of effector cells through the ligand-receptor interaction [37]. These data provide clues that strategies to block the PD-L1/PD-1 axis in HCC canboost up the tumor-specific immunity[38].

Another important effector subset of T helper cells is the follicular T-helper cells (Tfh). These are important to B cells during germinal centre reactions in secondary lymphoid tissues and function to support B-cell activation, affinity maturation, and isotype switching, leading to the generation of memory B cells and long-lived plasma cells[39]. Although, only a few studies have focused on humoral immunity in HCC and the regulatory mechanisms, an impairment of CD4+ Tfh cells have been indicated to influence the development of HBV associated HCC[40]. A decreased proportion of CXCR5+CD4+ Tfh cells was found to be associated with HCC disease progression. Furthermore, these cell types were found to have attenuated functioning with reduced secretion of IL-21 along with inability to promote B cell maturation and hence were suggested to be associated with low survival rates in HCC[41].

*Regulatory T lymphocytes*

Besides the anti-tumor cells that get functionally impaired during various cancers, there is another class of cells, termed regulatory T cells (Tregs) that express CD25 on their surface along with intracellular transcription factor, [forkhead](http://en.wikipedia.org/wiki/Fork_head_domain) box P3 (Foxp3), that have also been reported to play very important role in carcinogenesis[42, 43]. Under normal physiological conditions, natural Tregs (nTregs) limit autoimmune reactions by suppressing the self-reactive immune cells and are engaged in sustaining immunological self-tolerance and homeostasis.

It has been demonstrated in many studies that the number of a class of Tregs, called induced Tregs (iTregs), increase in peripheral blood as well as among the tumour infiltrating lymphocytes (TILs) of patients with HCC[44]. Depletion of Tregs led to the manifestation of anti-tumor immune responses in this study in around 38% of HCC cases[45]. While original investigations have only demonstrated an increase in the frequencies of Tregs in patients with HCC[46], ensuing research was focused on the possible correlation of Tregs with disease progression and the clinical outcome of disease in patients[47]. It has been reported that the number of Tregs correlated with disease severity, as the patients with advanced stages of HCC demonstrated a higher percentage of intra-hepatic CD8+Foxp3 regulatory T cells than patients in initial stages, suggesting that CD8+Foxp3+ regulatory T cells as well represent another immune-escape mechanism.. Moreover, there was reduced infiltration of CD8+ T cells in tumour, consequent to the abundant accumulation of Tregs in these areas as compared to non-tumour regions [48]. It has further been reported in another study that the FoxP3+ Tregs were highly amassed as activated cells, expressing CD69 and HLA-DR (terminally differentiated subpopulation), in the tumours where they could suppress the T-cell proliferative capabilities and IFN-γ secretion by T cells [48]. Hence it is suggested that the increased number of tumour-infiltrating Tregs foster tumour progression and serve as a poor prognostic marker in HCC patients.

Furthermore, Tregs through their membrane-bound TGF-β could also dampen the NK cell responses by down-regulating the NK group 2 member D (NKG2D) expression and participate in HCC progression [49]. Tumor-induced Treg (iTreg) seem to differentially regulate the NK cell activity in the tumor microenvironment and are also endowed with abilities to modulate T-cell proliferative abilities and functions of DCs via the anti-inflammatory cytokines like IL-10 and TGF-β. In contrast with the nTregs, tumor iTreg cells interfere with NK cells activated with IL-2, while IL-2 independent activation of NK cells was augmented in the presence of iTregs [50].

*Th17 cells*

Ever since it is known that tumour cells of HCC, TAMs and MDSCs, all are capable of producing adequate quantities of IL-6 and TGF-β, it can very well be speculated now that differentiation of Th17 cells in such an environment would be favoured, especially in established tumour tissues. Coupled with extreme inflammatory conditions in the growing tumour, an increased frequency of Th17 cells was eminent in the HCC tissues than in the non-tumour tissues, which positively correlated with the micro vessel density, a marker of tumour angiogenesis in tissues associated with poor endurance in patients with HCC[51]. Despite the positive correlation of Th17 cells with reduced survival of HCC cases, the roles of these cells in HCC still remain incompletely defined. Some studies have recently suggested that the IL-17 plays a dual role in tumour immunology. It can either promote anti-tumor cytotoxic T cell responses or can foster angiogenesis of surrounding endothelial cells and fibroblasts facilitating tumor growth[52]. In HCC patients, increased levels of Th17 and Th1 cells were observed in the tumor regions as compared to non-tumor regions and the frequency of these cells was associated with overall disease-free survival[53]. Thus, an elevated Th17 to Th1 ratio may promote tumor progression and may serve as a prognostic marker the same time.

More recent studies have shown that an imbalanced proportion of Th17 cells and Tregs is also associated with cancer progression, but not much is known about the implication of this disproportion in case of HCC[54]. The density of liver infiltrated FoxP3+ Tregs increased gradually from chronic hepatitis B infection to patients with atypical hyperplasia, then to HCC, while the density of Th17 cells and CD8+T cells in these cases trended towards a decrease as the disease progressed to HCC. In less differentiated HCC cases, the population of tumor-resident Tregs was present lower in number, while the percentages of Th17 cells and CD8+ T-cells were significantly greater. These findings indicate that Th17 cells and Tregs cooperate in the liver niche, thereby promoting the cancer advancement.

*NKT cells*

Natural killer T (NKT) cells are a subset of T lymphocytes that have overlapping properties with both T-cells as well as NK cells, expressing both αβ T-cell receptor along with many receptors of NK cells and are a potent source of cytokines like IL-4, IFN-γ and TNF-α. Depending on the diversity and extent of cytokines produced, their effects could be either beneficial or deleterious to the host. These cells recognize non-polymorphic molecule CD1d, through which the self and foreign lipid antigens are presented to them. These typical NKT cells, known as invariant NKT cells, act like a double-edged sword in case of cancer, promoting anti-tumor response on one hand by activating the effector cells and boosting the suppressor cell compartment on the other hand, thereby inducing tolerance[55].

Even though NKT cells constitute a major population in the liver, their role in hepato-carcinogenesis yet remains to be completely understood. The frequencies of NKT cells were increased in tumours, especially in patients with HCC with a gradual increment from blood to liver to tumour. A subset of these cells characterized by CD4 expression has been shown to get accumulated in the tumour environment and is able to generate Th2 cytokines that inhibit the tumour specific CD8+ T-cell response[56], while the other subset, CD4-NKT cells has anti-tumour effects and constitute a key role in dampening the inflammatory response mediated by the β catenin-driven hepatocarcinogenesis[57].

**Role of cytokines and chemokines**

*Dysregulated cytokine milieu*

Hepatocytes express receptors for several cytokines, thus making them susceptible to their action. Consequently, cytokines are involved not only in optimum functioning of liver, development and regeneration, but may also aid in the pathogenesis of liver cirrhosis, fibrosis and HCC. The cytokine *milieu* in livers with metastatic HCC is skewed towards a Th2 profile with an increase in levels of anti-inflammatory cytokines and a concomitant reduction in the pro-inflammatory cytokines. This also highlights the importance of Th1-type immune response in inhibiting tumor relapse[58].

*Th1 and Th2 cytokines*

The levels of Th1 cytokine, IL-2 is shown to have a direct correlation with prognosis in HCC patients as the increased levels of IL-2 were associated with an increase in the number of CD8+ T-cells[59]. Similarly, other Th1 cytokines like IFN-γ, IL-8, IL-15 and IL-18 have been indicated to be correlated with invasiveness and metastasis during HCC[60]. Alterations in these cytokines may help to control or ameliorate carcinogenesis as they are capable of changing the functional status of may cells like NK cells and cytotoxic T lymphocytes[61].

 The levels of Th2 cytokines, IL-4 and IL-5 were found to be high in the tumor microenvironment of metastatic HCC in patients with HBV-positive metastatic HCC, showing a shift from Th1 to Th2 profile[21]. The causative factor associated with switching of the cytokine balance is unknown, but factors produced by the tumor or by the microenvironment might play a role in tumorigenesis by polarizing cytokine production towards a Th2 phenotype.

Another cytokine released by Th22 cells [62], IL-22 has been found to be significantly elevated in HCC patients suggesting its involvement in T-cell-mediated immunity in HCC. A direct relationship between the levels of IL-22 and IL-17 in HCC patients indicate their interplay in the pathogenesis of HCC [63].

*Pro-inflammatory and Anti-inflammatory cytokines*

Tumor necrosis factor alpha (TNF-α) is an important mediator of inflammatory and autoimmune diseases and  is strongly involved in the pathogenesis of HCC, promoting invasion, angiogenesis, and metastasis [64]. In many cancers, including HCC, the serum levels of TNF-α has been reported to be very high, that correlated with disease status and nutrition status in these patients [65, 66]. Although in solid tumors, the levels of TNF-α were higher in normal tissues than in tumor cells, the serum levels were found to be lower in patients with HCC. Because of this discrepancy, the precise impact of cytokines associated with liver cancer development remains unclear [67]. TNF-α is also known to stimulate the expression of the negative co-stimulatory molecule B7 homolog 1 (B7-H1) or PDL-1 on macrophage surfaces, thus suppressing CD8+ T-cell antitumor immune response[38]. The downstream principal mediator of pro-tumoral activity of TNF-α is NF-κB, whose target genes are involved in cell proliferation and survival [68]. Of note, TNF-α is also induced by NF-κB in a positive feedback loop.

A higher production of IL-1β may help increase the production of other cytokines such as IL-2, IL-6, and TNF-α and trigger the complex immunological processes to eliminate the virus in case of Hepatitis induced HCC. Interestingly, besides its major role as a pro-inflammatory cytokine, IL-1β has been implicated as an important factor for tumor growth. Several independent lines of evidence have also suggested that genetic polymorphisms within IL-1β gene are associated with gastric cancer and HCC induced by HCV infection [69, 70]. Moreover, supplementing cytokines like TNF-α, IL-1β or IL-18 has been shown to induce growth of CD8+ T cells and also induce TRAIL in many HCC cell lines, contributing to tumor evasion [71].

The most studied anti-inflammatory cytokine in HCC is IL-10, which has been shown to be increased in HCC tumors versus non tumorous tissue adjacent to the tumor and tissues of healthy cohorts, respectively[72]. These studies suggest that increase in IL-10 in conjunction with other Th2 cytokines correlate with progression. Another multifunctional inflammatory cytokine, IL-6, which is produced mostly by resident macrophages was found to be linked with a poor prognosis of HCC patients[73]. IL-6 exerts its oncogenic activity by triggering downstream Signal Transducer and Activator of Transcription 3 (STAT-3) and Extracellular-signal-Regulated Kinases (ERK) pathways, which in turn control target genes involved in both cell proliferation and survival. It has been found that IL-6 levels and also its receptor expression were raised in a number of cancers, including HCC, where it may add onto tumor progression [74]. Recently, in a study carried out to investigate the use of novel serum biomarkers for predicting the recurrence and survival of patients with HBV-related HCC, a low serum IL-6 level, in addition to low platelet count and low serum albumin level, were found to be independent prognostic factors for disease free survival in these patients [75]. A recently recognized anti-inflammatory cytokine IL-37 has been shown to suppress cells of the innate immune system[76]. The study indicate that in HCC specimens, the expression of IL-37 was found to be decreased in tumour tissues and its expression levels were negatively related to tumour burden and related to improvement in survival.

Hence, it could be concluded that cytokines regulate the microenvironment of immune cells with allied and opposing roles, involving different signalling pathways to affect the course of HCC disease.

*Chemokine ligand-chemokine receptor axis*

Chemokines are known to direct the lymphocyte recruitment into liver tumors expressing the corresponding chemokine receptors [77]. The CXCL12-CXCR4 axis is regarded to be critical as a factor regulating tumor growth and progression during HCC. Previous studies have depicted higher expression of the CXCL12 and CXCR4 in HCC specimens, than the surrounding tissues[78]. It has been demonstrated in different studies that CXCR4 and CXCL12 may play a significant part in the HCC metastasis and invasiveness of the tumor[79, 80]. A significant correlation was observed between CXCR4 expression and tumor progression, metastasis and decreased survival rate[80]. However, no effect of loss of function mutation of tumor suppressor gene, p53 gene on CXCR4 expression in HCC, indicated yet another unidentified mechanism [81].

The ambiguity however exists on whether CXCR4-CXCL12 actually promotes the tumour growth as a down-modulation of CXCR4-CXCL12 expression in HCC both *in vitro* as well as *in vivo* has been reported where CXCL12/CXCR4 also lacked an association with death and HCC recurrence[82]. Therefore, although it appears that the CXCL12-CXCR4 axis is indispensable in HCC, yet its precise role still remains paradoxical in this disease. Possible involvement of another important axis CCL20-CCR6 axis in HCC has been suggested because of significantly upregulated expression of both CCL20 and its chemokine receptor, CCR6 have been observed in HCC tissues that varied with different rates of tumour progression[83]. The role of Fractalkine (CX3CL1) and its receptor CX3CR1 in HCC although indicated role in regulation of immune responses, yet the relationship of fractalkine-CX3CR1 axis with HCC is not clear so far. According to recent studies, the fractalkine-CX3CR1 axis is critical in the diagnosis of HCC, as it can regulate both the immune response and the cell cycle of HCC [84].

Furthermore, the expression levels of some chemokine receptors like CCR5, CCR6, and CXCR3 on the surface of peripheral lymphocytes of HCC patients was reduced, while the expression of these receptors on tumor-infiltrating cells was higher suggesting a role of these chemokine receptors in controlling the trafficking of effector T cells to the tumor regions in response to the corresponding chemokines[85]. In addition to this, the expression levels of CXCR3 have been reported to be particularly higher on tumor infiltrating cells as compared to non-tumor infiltrating cells, implying, that lymphocytes preferentially migrate to the tumor tissue rather than the surrounding non-tumor regions. This increased expression was negatively correlated with tumor burden and the stage of cancer. The literature citing the role of various immune components in HCC is summarized in **Table 1**.

**Table1: Summary of the status of various immune components in HCC**

|  |  |  |
| --- | --- | --- |
| **Immune component** | **Status in HCC** | **References** |
| Dendritic cells | Decreased antigen presentation, decrease in numbers, Impaired functions | [12, 13] |
| Macrophages | Poor antigen presentation, activate Th2 immune responses, promote Tregs | [17, 18, 20] |
| Myeloid derived suppressor cells | Exert suppressive functions through free radicals, arginase activity and TGF-β | [21, 22] |
| Neutrophils | Promote angiogenesis through metalloproteinase-9 | [24] |
| NK cells | Decrease in numbers, low cytolytic activities | [26, 28] |
| T lymphocytes | Decrease in frequencies, Less Th1 cytokines, Increased expression of inhibitory receptors | [36, 37] |
| Regulatory T cells | Increase in frequencies, suppress T-cell proliferation and IFN-γ secretion, Inhibit NK cell responses | [42, 48, 86] |
| Th17 cells | Increase in numbers, Role incompletely defined, correlate with disease progression | [51, 52] |
| NKT cells | Dual roles, Increased frequencies, promote Th2 cytokines | [55, 56] |
| Th1 cytokines | Decreased in tumor microenvironment, induce CD8+ T cells | [59, 61, 87] |
| Th2 cytokines | Increased levels, Correlation with tumor progression,  | [21] |
| Proinflammatory Cytokines | Involved in pathogenesis of HCC | [65, 69] |
| Anti-inflammatory Cytokines | Increased in HCC, correlate with progression | [72, 73, 76] |
| Chemokine-receptor axis | Tumor progression and metastasis | [78, 83, 84] |

**Gaps in existing Knowledge**

 Insights into the immune signalling pathways are being provided by recent studies analysing the role of immune effector cells. Still, complete understanding of many immune components such as NKT cells, gamma delta T cells and the role of many cytokines and chemokines is not achieved so far.  It is generally believed that T lymphocytes play a protective role in inhibiting tumor growth and development while TAMs, MDSC, Tregs and Th17 cells and their associated cytokines IL-6, TNF-α, IL-1β, IL-23 and TGF-β may play important roles in promoting the growth and survival of cancer. However, defining their roles as pro-tumor or anti-tumor needs caution. It is also unclear how TAMs and TGF-β regulate the generation and function of Tregs in the developing and established solid tumor microenvironment. Of further importance is understanding whether TGF-β production, preferentially induces Tregs, or instead promotes the development of Th17 cells within the microenvironment of tumors. Further research into better understanding of the balance between all immune components at all stages of carcinogenesis is essential for the development of effective cancer therapies that would target or utilize immunological mechanisms.

 Recent observation in many solid tumors suggests the use of checkpoint inhibitors (that decide a balance between co-stimulatory and inhibitory signals) in inducing strong anti-tumor response that needs to be evaluated in HCC also. The choice of a therapeutic agent to target various checkpoints along with tumor vaccines represents novel strategies to induce immune resistance. These combinatorial approaches will induce tumor regression in patients that would not have responded to either of the treatment alone. Strategies to deliver genetically modified T cells into the tumor microenvironment such as into a hepatic artery are underway and being evaluated in clinical trials that have already proven successful in the treatment of other cancers [88]. Novel epitopes specific for specific tumor associated antigens should be designed using high throughput ''omics'' technologies that would aim to induce anti-tumor CD4+ and CD8+ T cell responses. In this context, high resolution mass spectrometry has been used for directly sequencing peptides presented by HLA molecules from tumor cells so as to identify naturally processed class I and II tumor-associated peptides[89]. Combining key components of the tumor microenvironment as compared to chemotherapy alone would improve the clinical outcome. Finally, therapeutic agents that are capable of reversing the immunosuppressive nature of HCC tumors, administered alone or in combination with other modalities, will be critical in optimizing the clinical outcomes for HCC patients.

**Concluding remarks**

Since HCC accounts for 90% of all liver cancers and is usually multifocal at the time of diagnosis, it makes treatment difficult and is affronted with a higher recurrence rate in these patients. The incidence is accelerating at a regular rate and will likely increase in the time to come. Hence, in this context, there is an imperative demand for newer and better therapeutic strategies to combat this predicament. This needs a fuller discernment of the function of various components of our immune system and how they interplay in shaping up the immune responses against the tumor. Immune suppression is predominantly mediated by the cytokine secreted in the local *milieu* by Tregs that down-regulate the effector and cytotoxic activities of CD8+ T cells and NK cells. The antigen presenting functions of DCs are also affected due to the expression of several inhibitory receptors on them that further suppress the helper T cell functions. The TAMs and MDSCs contribute to the ongoing inflammation and participate in the activation of a Th2 immune response favouring Treg recruitment and development, thus promoting angiogenesis. These cell types can also help in the differentiation of Th17 cells that also infiltrate the tumor micro-environment and correlate with poor survival in HCC patients, however, their roles still remain incompletely defined. Similarly, NKT cells despite being predominant population in the liver, their role in hepatocarcinogenesis remains to be completely elucidated. The soluble factors including cytokines and chemokines play a crucial role in immune-surveillance and immune-regulation. The cytokine *milieu* in livers with metastatic HCC is skewed towards a Th2 profile with a concomitant decrease in the pro-inflammatory cytokines. The role of many cytokines like IL-22, have recently been deciphered in HCC which adds on to the current knowledge about the *milieu* of liver tumor. The chemokine ligand-chemokine receptor axis plays role in regulating the differential recruitment of effector T cells to the tumor and the interconnections between different axes and not just a single axis is desired to be surmised. Future studies are warranted to understand the complexity of interactions between these immune cells to potentiate the immune system and for designing of newer immune-therapeutics against HCC.

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