

# Regulatory B cells: key players in hepatocellular carcinoma progression

## Abstract

Hepatocellular carcinoma (HCC) represents a major health problem and ranked as the sixth most common cancer worldwide and the third most common cause of cancer-related mortality. The prognosis of HCC is usually poor due to post surgery recurrence and metastasis. Both Cells mediated and humoral immunity is considered as key players in the immunopathology of HCC. Recently, there is a special subset of B cells defined as regulatory B cells (Bregs) found to be abundant in the tumor microenvironment and was a leading cause of progression of various cancers, including HCC.

Bregs arise from a common progenitor named transitional 2 marginal zone precursor (T2-MZP) B cells as they have most of the indicated markers for Bregs. Human Bregs or also known as human IL-10 producing B cells (B10) is a subset of B cells is enriched in the CD19<sup>+</sup>CD24<sup>high</sup>CD27<sup>-</sup>CD38<sup>high</sup>CD1d<sup>high</sup>CD5<sup>+</sup> transitional B cell subset. Tumor-Evoked Regulatory B Cells (tBreg) exert antitumor activity by promoting conversion of the resting CD4<sup>+</sup> T cell into FoxP3<sup>+</sup> Treg by secretion of TGF- $\beta$  then the Treg inhibit T cell proliferation and promote tumor metastasis by suppression of the anti-tumor effects of CD8<sup>+</sup> T cells and NK cells.

Bregs may suppress the antitumor immunity and promote HCC progression via several mechanisms including the CD40/CD40L signaling-mediated cytokine production of IL10, TGF- $\beta$  which down regulate TNF- $\alpha$ , PD-1<sup>hi</sup> B-cell, Granzyme B secreting B cells (GrB<sup>+</sup> B cells), Treg upregulation, TH17 downregulation and IL35 which triggers the genesis of Tregs from naive T cells with subsequent suppression of the anticancer immune response. The hallmark of Breg function is IL-10, which inhibits proinflammatory cytokines and supports Treg differentiation. In this review, we highlight the role and mechanisms by which Breg cells are involved in HCC. Understanding these mechanisms may direct us to a novel therapeutic approach targeting Breg for treatment of HCC.

**Keywords:** Hepatocellular carcinoma; Breg; tBreg; IL10 and immune regulation

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**Abbreviations:** HCC:Hepatocellular Carcinoma, Breg:Regulatory B Cell, Treg:Regulatory T Cell, IL:Interleukin, CTL:Cytotoxic T Lymphocytes, tBreg:Tumor Evoked Regulatory B Cells, GrB<sup>+</sup> B cells:Granzyme B Secreting B Cells

## Introduction

Hepatocellular carcinoma (HCC) is the most common histologic type of primary liver cancer. HCC represents a major health problem and ranked as the sixth most common cancer worldwide and the third most common cause of cancer-related mortality.<sup>1</sup> The prognosis of HCC is usually poor due to post surgery recurrence and metastasis.<sup>2</sup> There is a strong relationship between immune system and cancer progression or regression. The immune system could specially identify tumor cells through the tumor-associated antigens (TAA) and eliminate them and this process described as tumor immune surveillance.<sup>3</sup>

Both Cells mediated and humoral immunity is considered as key players in the immunopathology of HCC. Cancer cells are recognized and eliminated by the innate immune system such as nature killer cells (NK) and macrophages then by the adaptive immune response mainly by Cytotoxic T lymphocytes CTL before they became clinically apparent. The tumor cells can escape from the immune system by decreasing the expression of tumor-specific antigens and even lose the major MHC-I and MHC-II antigens and so the tumor cells became less immunogenic, escaped from immune attack, and

suppressed antitumor immune response leading to tumor growth and genesis resulting from the immune evasion and escape.<sup>2</sup>

Recently, many reports explored new subsets of the immune cells as regulatory T cells (Tregs) and regulatory B cells (Bregs) which are linked to the tumor immune surveillance and suppress the immune system to promote cancer genesis and progression.<sup>2,4</sup>

Bregs are special subset of B cells found to be abundant in the tumor microenvironment and was a leading cause of progression of various cancers, including HCC. Breg induce progression of HCC by several signaling pathways.<sup>4,5</sup> In this review, we will highlight the role and mechanisms by which Breg cells are involved in HCC. Understanding these mechanisms may direct us to a novel therapeutic approach targeting Breg for treatment of HCC.

## Origin and characteristics of Regulatory B-cells

In 2002, Mizoguchi and collaborators introduced the term "regulatory B cells" and identified Bregs as an IL-10-producing B cell subset.<sup>6</sup> Breg cells have been identified as a negative regulator of the immune system that inhibit pathological immune response by suppressing both uncontrolled protective immune response and damaging autoimmune responses. The mechanism by which Bregs suppress inflammatory responses is mainly via the production of IL-10.<sup>7</sup> Mauri et al.<sup>8</sup> reported that Bregs arise from a common progenitor named transitional 2 marginal zone precursor (T2-MZP) B cells.<sup>9</sup> as they have most of the indicated markers for Bregs.<sup>8</sup> Human

regulatory B-cells or also known as human IL-10 producing B cells are a subset of B cells is enriched in the CD19<sup>+</sup> CD24<sup>high</sup>CD27<sup>-</sup>CD38<sup>high</sup>CD1d<sup>high</sup>CD5<sup>+</sup> transitional B cell subset. Additionally, these B cells are highly enriched in IL-10 expressing B (B10) cells.<sup>10</sup> In the presence of toll like receptor (TLR) ligands, the inflammation cascade initiated and B cells receive BCR, CD40, or CD80/CD86 activating signals leading to release IL-10.<sup>8,11</sup> IL-10 plays an essential role in inducing immunoregulatory phenotype of B cells that exert massive anti-inflammatory and immunosuppressive actions.<sup>12</sup>

- i. **B cells in cancer:** B lymphocytes could have both positive and negative role during antitumor immune response.<sup>13</sup> B cells may cause tumor regression through IgG 2b-dependent manner and by facilitating T cell mediated responses.<sup>14</sup> On the other hand, B cells could have a negative regulatory role during immune response against tumors as they can promote tumors by secretion of TGF- $\beta$ 1 that suppresses the antitumor cellular immune responses. B cells secrete IL10 and TGF- $\beta$  that mediate TH2 activation and subsequent suppression of the cytotoxic activity of CD<sup>+</sup> T cells.<sup>13,14</sup> B cells secrete cytokines and antibodies that act via FcR and complement to mediate chronic inflammation that promote carcinogenesis.<sup>15</sup> Also, the antibodies secreted from B cells form immune complexes that stimulate myeloid cells recruitment to tumor microenvironment and the myeloid cells promote tumor growth by binding to the Fc $\gamma$ -activating receptors.<sup>15,16</sup>
- ii. **Breg cells and cancer:** The role of Bregs in cancer had been highlighted when recent studies explored that IL10 regulate the tumor inhibition effect of T cells. IL10 is regulatory cytokine that can inhibit the expression of TH1 and TH2 cytokines. Breg could exert their regulatory function by secretion of cytokines and antibodies which promote immune complex production and stimulate signals that lead to tumor progression. Breg secrete IL10 and TGF- $\beta$ 1 which inhibit the cytotoxic activity of TH1/CD8<sup>+</sup> T cells and this promotes tumor growth.<sup>4,5,17</sup>
- iii. **Tumor-Evoked Regulatory B Cells (tBreg) and cancer:** Biragyn et al.<sup>18</sup> explored a novel population of B cell subsets designated as Tumor-Evoked Regulatory B Cells (tBreg).<sup>18</sup> Interestingly, tBregs represent a functionally and phenotypically unique subset of B cells. Functionally, tBreg can promote conversion of the resting CD4<sup>+</sup> T cell into FoxP3<sup>+</sup> Treg by secretion of TGF- $\beta$  then the Treg inhibit T cell proliferation and promote tumor metastasis by suppression of the anti-tumor effects of CD8<sup>+</sup> T cells and NK cells. Interestingly, cancer cells themselves might inhibit the anticancer immune responses by converting normal B cells into tBreg.<sup>18,19</sup>

Phenotypically, tBregs resemble activated but poorly proliferative mature B2 cells (CD19<sup>+</sup> CD25<sup>High</sup> CD69<sup>High</sup>) that express high levels of active STAT3. In case of absence of a unique surface marker, tBregs can be described as CD19<sup>+</sup> B cells that are CD25<sup>High</sup> B7-H1<sup>High</sup> CD81<sup>High</sup> CD86<sup>High</sup> CCR6<sup>High</sup> and CD2L<sup>Low</sup> IgM<sup>Int/Low</sup>.<sup>18,20</sup>

## Breg cells and HCC

Bregs with CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> phenotype was reported to be enriched in the tumor microenvironment and found to be associated with progression of several tumors including HCC. Bregs promote HCC progression via the CD40/CD40L signaling-mediated cytokine production of IL10, TGF- $\beta$  and TNF- $\alpha$ .<sup>4,5,17</sup>

Shao et al. studied the clinical association of Breg with HCC and explored the mechanisms by which Breg interact with HCC and concluded that there is a positive correlation between HCC

progression and both the frequency of intra hepatic Breg at the tumor margin and the peripheral Bregs.<sup>5</sup> Peripheral Bregs could migrate from the circulation and home to the liver and accumulate at the tumor margin in HCC to promote tumor progression and invasion so a postoperative follow up of the dynamic change in the peripheral Breg is recommended to predict the possibility of recurrence in HCC patients.<sup>5</sup>

Breg induce the invasion and proliferation of HCC in vivo and in vitro models by direct interaction with HCC through the CD40/CD154 signaling pathway and release of IL10 and TGF- $\beta$ 1 that decrease the release of TNF leading to HCC progression because TNF- $\alpha$  is critical for antitumor immunity.<sup>5,21,22</sup>

Depletion of Breg or blockage of CD40/CD154 interaction between Breg and HCC cells might be a future novel therapeutic approach in treatment of HCC because inhibition of this pathway will decrease the secretion of IL10, TGF- $\beta$ 1 but increase in the level of TNF- $\alpha$  which inhibit tumor growth.<sup>5</sup>

It had been shown that peripheral and intra tumoral Bregs and Tregs are critical in the pathogenesis of HCC. Chen et al studied the perioperative dynamic change in the frequency of circulatory Tregs and Bregs in HCC patients and they reported that the frequency of peripheral Bregs and Tregs were low before surgery and significantly elevated after resection.<sup>2</sup> The postoperative increase in the Tregs and Bregs in HCC patients might suppress the host immune responses and promotes tumor metastasis and recurrence. HCC may produce IL8,<sup>23</sup> and CCL20,<sup>24</sup> that induce homing of peripheral Bregs and Tregs to the tumor microenvironment to suppress the anticancer immune responses.<sup>25</sup>

There is strong evidence that Breg interact with Treg in the tumor microenvironment: Breg may induce the conversion of resting CD4<sup>+</sup> T cells into Treg to support tumor progression and metastasis by suppressing the T cell anticancer immune response.<sup>25</sup> Bregs which promote Tregs are characterized by the expression of high level of STAT3 and B7-H1.<sup>18</sup> and this is matched with the finding that the expression of IL10 and B7-H1 was upregulated in HCC microenvironment which support the concept that Bregs promote Tregs in HCC microenvironment to support tumor progression.<sup>2</sup> Previous studies have reported that immunotherapy against Treg might enhance anticancer immunity.<sup>26</sup> so, postoperative strategy against Bregs and Tregs may be a novel therapeutic approach to improve prognosis of post-surgery HCC patients and to decrease the chance of metastasis and recurrence.<sup>2</sup>

B lymphocytes have a regulatory effect on the antigen-presenting function of DCs. Bregs interact with DCs to promote IL-4 secretion, possibly by downregulating their secretion of IL-12 released by DCs, thereby leading to priming of both Th1 and Th2 lymphocytes and favoring the induction of a nonpolarized immune response.<sup>27</sup> The dysfunction of DCs and the high frequency of IL-10-producing Breg and Foxp3<sup>+</sup> Treg might play important roles in HCC progression.<sup>28</sup>

Breg cells can do their regulatory function in HCC by several indirect and direct mechanisms. Bregs promote HCC progression and invasion via the CD40/CD40L signaling pathway of cytokine production of IL10, TGF- $\beta$  and TNF- $\alpha$ .<sup>4,5,17</sup>

**IL10 and TGF- $\beta$ 1:** TNF- $\alpha$  is a proinflammatory cytokine crucial for antitumor activity and the decrease in its level was reported to be associated with HCC development and progression. Breg secrete IL10 and TGF- $\beta$ 1 which might promote tumor growth by downregulation of TNF- $\alpha$  expression.<sup>5,29</sup> Moreover, the production of TNF- $\alpha$  by

macrophage was reported to be suppressed by CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>h</sup> B reg cell phenotype.<sup>30</sup> IL10 can suppress the secretion of chemokines and proinflammatory cytokines with subsequent downregulation of the costimulatory molecules by antigen presenting cells APCs. Also, IL10 might downregulate the expression of CD86 leading to decreased TNF $\alpha$  production and inhibition of T cell proliferation.<sup>30</sup> While IL-10 is a major key player of Breg, there are other mechanisms of Breg cell immunosuppression that are IL-10 independent.<sup>10</sup>

**PD-1<sup>hi</sup> B-cell:** Recent study done by Xiao et al explored a novel protumorigenic subset of B cells named PD-1<sup>hi</sup> B-cell that express the programmed cell death-1 (PD-1) in a high level and represent about 10 % of the B cells present in the tumor microenvironment in patients with advanced stage of HCC.<sup>31</sup> PD-1<sup>hi</sup> B-cell is distinguished from the conventional peripheral Breg in that they exhibit a unique CD5<sup>hi</sup>CD24<sup>-/+</sup>CD27<sup>hi/+</sup>CD38<sup>dim</sup> phenotype that is different from the ordinary CD24<sup>hi</sup>CD38<sup>hi</sup> peripheral Breg phenotype.<sup>17,31,32</sup>

HCC environmental factors might induce the PD-1<sup>hi</sup> Breg cells through TLR mediated BCL6 upregulation and this induction could be inhibited by IL4-induced STAT6 phosphorylation.<sup>31</sup> When PD-1<sup>hi</sup> B-cell encounter PD-L1<sup>+</sup> cells or undergo PD-1 triggering: they exert regulatory functions that could effectively suppress tumor-specific T-cell immunity and promote tumor growth through IL10 signaling pathway.<sup>31</sup>

**Granzyme B secreting B cells (GrB<sup>+</sup> B cells):** Granzyme B is a cytotoxic protease expressed in CTL, NK cells, Tregs as well as plasmacytoid dendritic cells (pDCs) and exhibit cytolytic activity.<sup>33-35</sup> GrB<sup>+</sup> B cells express molecules that play a role in immune tolerance, as IL-10, CD25.<sup>33</sup> GrB<sup>+</sup> Bregs may be localized within the tumor microenvironment adjacent to IL-21-secreting Tregs that promote immune tolerance. IL21 induce the outgrowth of B cells expressing high levels of GrB that could inhibit T cell proliferation via GrB-dependent degradation of T-cell receptor (TCR)- $\zeta$  chain.<sup>36-38</sup> IL-21 may induce GrB<sup>+</sup> human Bregs that may infiltrate tumors and suppress anti-tumor immune responses.<sup>33</sup> On the other hand, IL-21 might induce expression of GrB in B cells which may exert cytotoxic activity against tumor cells.<sup>39</sup>

**Cross talk with T-lymphocytes:** Breg secrete IL10 that can suppress proliferation and cytokine production by TH1 cell. Additionally, IL10 can convert effector T cells to Treg cells which suppress the tumor specific immune response. IL10 secreted by Breg can affect TH1/TH2 balance by promoting DCs to secrete IL4 and downregulate IL12.<sup>27</sup>

Additionally, IL10 secreting Bregs (B10 cells) might affect balance between FoxP3<sup>+</sup>Treg /TH17 via suppression of TH17 cell differentiation by reducing the STAT3 phosphorylation levels with subsequent decrease in the expression level of the retinoid-related orphan receptor  $\gamma$ t (ROR $\gamma$ t).<sup>40</sup> Also, there is a possibility that B10 cells might promote Treg differentiation by upregulating FoxP3 expression and this will ultimately lead to suppression of the anticancer immune response.<sup>40</sup> Recent studies reported that CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>h</sup> B reg promote activation and expansion of iNKT cells and might also convert effect or T-cell into Treg cells.<sup>41</sup>

Moreover, IL21 producing T-cell up regulate the expansion of IL10 producing B cells.<sup>42</sup> On another study, It was shown that follicular TH cells produce IL21 that increase differentiation of Breg cells.<sup>43</sup>

**InterleukinL35 (IL35):** IL35 is a regulatory cytokine that promotes tumor progression by suppression of the T cell anticancer response by

stimulating myeloid cell accumulation in tumor microenvironment and that inhibit CTL response and induce immune suppression.<sup>44</sup>

Treg cells secrete IL-35 that may induce naive T cells to convert into Tregs (iTr35 cells. Thus, IL-35 triggers the genesis of more Tregs that suppress the anticancer immune response.<sup>44</sup>

## Discussion

HCC represents the third most common cause of cancer-related mortality with bad prognosis of HCC is due to post surgery recurrence and metastasis. The patient immune response plays a crucial role in the immunopathology of HCC. Recently, Bregs found to be abundant in the tumor microenvironment and was a leading cause of progression of various cancers, including HCC. Bregs are characterized by the expression of CD19<sup>+</sup>CD24<sup>high</sup>CD27<sup>-</sup>CD38<sup>high</sup>CD1d<sup>high</sup>CD5<sup>+</sup>.<sup>9,10</sup> Bregs may suppress the antitumor immunity and promote HCC progression via several mechanisms including the CD40/CD40L signaling-mediated cytokine production of IL10, TGF- $\beta$  which downregulate TNF- $\alpha$  which is crucial for antitumor immunity.<sup>4,5</sup> Additionally, when PD-1<sup>hi</sup> B-cell encounter PD-L1<sup>+</sup> cells, they exert regulatory functions that suppress tumor-specific T-cell immunity and promote tumor growth through IL10 signaling pathway.<sup>31</sup> Moreover, Breg may act through Treg upregulation, TH17 downregulation and IL35 which triggers the conversion of naive T cells into Tregs which suppress the anticancer immune response.<sup>45</sup>

Recent reports showed that resveratrol (RSV), at low and non-cytotoxic doses for immune cells, can inhibit lung metastasis in mice. RSV inactivates Stat3, preventing the generation and function of tBregs, including expression of TGF- $\beta$  leading to inhibition of tBreg-mediated Treg conversion, therefore recover the ability of CD8<sup>+</sup> T cells and NK cells to exert anti-tumor responses with efficient inhibition of lung metastasis in mice.<sup>19</sup>

Breg depletion can be a useful adjunct in human immunotherapy. It has been reported that B-cell depletion using an anti-CD20 antibody was effective inhibitor of tumor growth in many solid tumor models and augmenting immunotherapy in a tumor vaccine model.<sup>20,46</sup>

## Concluding remarks

Bregs are involved in HCC progression and might be used as novel prognostic marker of HCC. Breg induce the invasion and proliferation of HCC in vivo and in vitro models by direct interaction with HCC through the CD40/CD154 signaling pathway and release of IL10 and TGF- $\beta$ 1 that decrease the release of TNF leading to HCC progression because TNF- $\alpha$  is critical for antitumor immunity. Depletion of Breg or blockage of CD40/CD154 interaction between Breg and HCC cells might be a future novel therapeutic approach in treatment of many kinds of solid tumors including HCC.

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