

# B10 cells: Development, phenotype, and function in cancer

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## Conflict of interest

None declared

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## Abstract

B10 cells, a specialized subset of regulatory B cells, have been identified in both mice and humans. These cells are characterized by their regulatory impact on immune dynamics, principally through their secretion of interleukin-10 (IL-10), a cytokine known for its anti-inflammatory properties. The pivotal role of immune mediators such as B10 cells is to maintain a delicate equilibrium between antitumor immunity and tumor-promoting responses. Emerging studies have cast B10 cells as key suppressors in the antitumor immune arsenal. They operate in synergy with a spectrum of immune cells within the innate and adaptive spectrums, contributing to a milieu that favors tumor progression and metastatic spread. In this comprehensive review, we will discuss the ontogeny, phenotype and effector functions of B10 cells in murine systems. We will also review the role of B10 cells in oncological models in animal studies and extend these findings to the human clinical context, elucidating their role in facilitating tumor immune evasion. A thorough understanding of these processes is imperative for the strategic targeting and attenuation of B10 cell activity, which is anticipated to be a cornerstone in the advancement of effective cancer immunotherapy strategies.

**Key words:** cancer, IL-10, tumor immunology, B10 cells

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## Introduction

B cells (Bregs) have undergone extensive research due to their diverse roles in antigen presentation, T cells activation, and secretion of antibodies, cytokines, and proteases.<sup>1–6</sup> B cell development originates from hematopoietic stem cells in bone marrow. Immature transitional B cells leave the bone marrow and circulate through the peripheral immune organs, where they further mature into naïve B cells. Upon stimulation by specific antigens, naïve B cells proliferate in lymphoid follicles and form the germinal center (GC). Here, activated B cells differentiate into short-lived plasmablasts that secrete antibodies, as well as long-lived plasma cells and memory cells. Each subset has distinct phenotypes, and they all function as effector cells in humoral immune responses (Table 1).<sup>7–13</sup> In recent years, many studies have described the role of B cells with immunosuppressive functions in regulating antitumor immune responses. Regulatory B cells were originally used by Mizoguchi et al. to refer to B lymphocytes that prevent inflammatory bowel disease (IBD) from progression in mouse models,<sup>14,15</sup> mainly by suppressing and/or modulating immune responses by producing the potent anti-inflammatory cytokine interleukin 10 (IL-10).<sup>16</sup> To date, the unique phenotype of B cells has not been determined, and for clarity, the subset of regulatory B cells with the function to express IL-10 is referred to as B10 cells. We use the term “B10 cell” to denote B cells capable of expressing IL-10 in vitro. Due to the ability of B10 cells to induce a tolerogenic environment and increase the number and activity of immunosuppressive cells, increasing evidence supports a role of B10 cells in tumor immunity to promote tumor progression.

## Objectives

In this review, we discuss the identification, distribution and development of B10 cells. We provide a summary of the functions of key surface molecules in B10 cells. Additionally, we analyze the phenotypes and functions of B10 cells in both human and animal models of solid

malignancies (Table 2). We also discuss therapeutic options that could be used to reduce B10 cell activity and boost antitumor reactions.

## Materials and methods

Data for this review were collected in January 2023. The search strategy involved searching the PubMed database using the keywords “B10 cells”, “regulatory B cells” and “IL-10-producing B cells”. During the 2<sup>nd</sup> round of data collection, more precise keywords were used: “development”, “function”, “toll-like receptors”, “cytokine”, “inflammation”, “tumor”, “T cells”, and “IL-10”. This more focused search produced 91 articles. Articles were selected based on their relevance to our objectives.

## Results

### Identification, distribution and surface markers

The identification of B10 cells was studied in mice using a contact hypersensitivity (CHS) model.<sup>16</sup> In this model, CD19<sup>-/-</sup> mice (B10 cells in the spleen: 1–2%) exhibited an increased T-cell-dependent inflammatory response resulting in ear thickness augmentation, compared to wild-type mice (B10 cells in the spleen: absent). Conversely, CD19 transgenic (CD19Tg) mice (B10 cells in the spleen: ~10%) exhibited reduced inflammation. Previous research has indicated that B cell-mediated IL-10 secretion ability parallels the CHS response inhibition in CD19<sup>-/-</sup>, WT and CD19Tg mice.<sup>16</sup> It has been shown that transplanting IL-10-producing B cells from wild-type mice into CD19<sup>-/-</sup> mice may decrease inflammation in the CD19<sup>-/-</sup> mice, and that this reduction is dependent on the generation of IL-10 in the B cells. In addition, CD19Tg mice showed aberrant B-1 subset development, altered antibody and autoantibody production, and alterations in the T-cell compartment.<sup>16</sup> Therefore, the production of the powerful inhibitory cytokine IL-10

**Table 1.** B cell subsets in mice and humans

Subsets	Mouse phenotype	Human phenotype	Reference
Transitional	CD19 <sup>+</sup> B220 <sup>+</sup> CD24 <sup>+</sup>	CD19 <sup>+</sup> CD24 <sup>hi</sup> CD38 <sup>hi</sup>	7
Mature naïve	FO-B: B220 <sup>+</sup> IgM <sup>lo</sup> IgD <sup>hi</sup> CD21 <sup>int</sup> CD23 <sup>+</sup>	CD19 <sup>+</sup> CD20 <sup>+</sup> CD27 <sup>+</sup> CD24 <sup>int</sup> CD38 <sup>int</sup> CD21 <sup>+</sup> CD10 <sup>-</sup> IgD <sup>+</sup> IgM <sup>-</sup>	8–10
	MZ-B: IgM <sup>hi</sup> IgD <sup>lo</sup> CD21 <sup>hi</sup> CD23 <sup>-</sup> CD1d <sup>hi</sup>		
Germinal center	CD19 <sup>+</sup> CD38 <sup>lo</sup> PNA <sup>+</sup> GL7 <sup>+</sup> FAS <sup>+</sup>	CD19 <sup>+</sup> CD10 <sup>+</sup> CD20 <sup>+</sup> CD27 <sup>+</sup> CD38 <sup>+</sup> FAS <sup>+</sup> IgD <sup>+</sup> DBR3 <sup>+</sup>	10, 11
Memory	CD19 <sup>+</sup> B220 <sup>+</sup> CD38 <sup>+</sup> and/or CD80 <sup>+</sup> and/or PD-L2 <sup>+</sup> and/or CD73 <sup>+</sup>	CD19 <sup>+</sup> CD27 <sup>+</sup> CD38 <sup>-</sup>	7
Antibody-secreting cells	plasmablasts: CD19 <sup>lo/-</sup> CD138 <sup>+</sup> CD93 <sup>+</sup> MHCII <sup>+</sup> Ki-67 <sup>+</sup>	plasmablasts: CD19 <sup>+</sup> IgD <sup>-</sup> CD27 <sup>hi</sup> CD38 <sup>hi</sup> CD138 <sup>-</sup>	12, 13
	plasma cells: CD19 <sup>lo/-</sup> CD138 <sup>+</sup> CD93 <sup>+</sup> MHCII <sup>+</sup> Ki-67 <sup>+</sup>	plasma cells: CD19 <sup>+</sup> IgD <sup>-</sup> CD27 <sup>hi</sup> CD38 <sup>hi</sup> CD138 <sup>+</sup>	

FO-B – follicular B cells; MZ-B – marginal zone B cells.

led to the functional definition of this distinct regulatory subgroup as the B10 cell. Because of their small population size, IL-10-producing B cells (also known as “B10 effector cells”) can be difficult to detect *in vivo*. However, the ability of B10 effector cells (B10eff) to produce IL-10 can be tested *in vitro*. B10 cells can be detected using flow cytometry after being stimulated with PMA (phorbol 12-myristate 13-acetate), ionomycin and monensin (PIM) for 5 h *ex vivo*.<sup>16–18</sup> In addition, increased IL-10 production after co-culturing B10 with lipopolysaccharide (LPS) or CpG compared to stimulation with PMA and ionomycin alone suggests that B10 are more likely to be antigen-experiencing cells.<sup>19–21</sup>

B10 cells comprised between 1% and 2% of spleen B cells and over 40% of peritoneal cavity B cells of wild-type mice.<sup>16</sup> These cells are also detected in various tissues, including blood, lymph nodes, Peyer’s patches, intestinal tissues, and the central nervous system, albeit at extremely low levels.<sup>19,22,23</sup> When compared to non-B10 cells, B10 cells from untreated mice exhibit elevated levels of immunoglobulin M (IgM), CD19, CD5, CD24, CD43, and MHCII, along with reduced IgD and CD23 expression.<sup>23</sup> Phenotypically, spleen B10 cells resemble various distinct B cell subsets and can be further categorized based on cell surface marker expression into transitional cells, marginal zone (MZ) cells, marginal zone precursor cells, and memory cells.<sup>16,24–26</sup> The specific marker for spleen B10 cells is CD19<sup>hi</sup>CD1d<sup>hi</sup>CD5<sup>+</sup>.<sup>16,24,27,28</sup> However, B10 cells also occur in low frequencies among other spleen B cells populations, such as those marked CD1d<sup>lo</sup> and CD5<sup>-</sup> population B cells.<sup>29</sup> B10 cells from the peritoneal cavity and spleen share similar phenotypes, despite the absence of CD1d<sup>hi</sup> or CD21<sup>hi</sup> B cells in the peritoneum.<sup>23</sup> Based on surface molecule expression, peritoneal cavity B10 cells can be further subdivided into B1a (CD5<sup>+</sup>CD11b<sup>+</sup>) and B1b (CD5<sup>-</sup>CD11b<sup>+</sup>).<sup>23</sup>

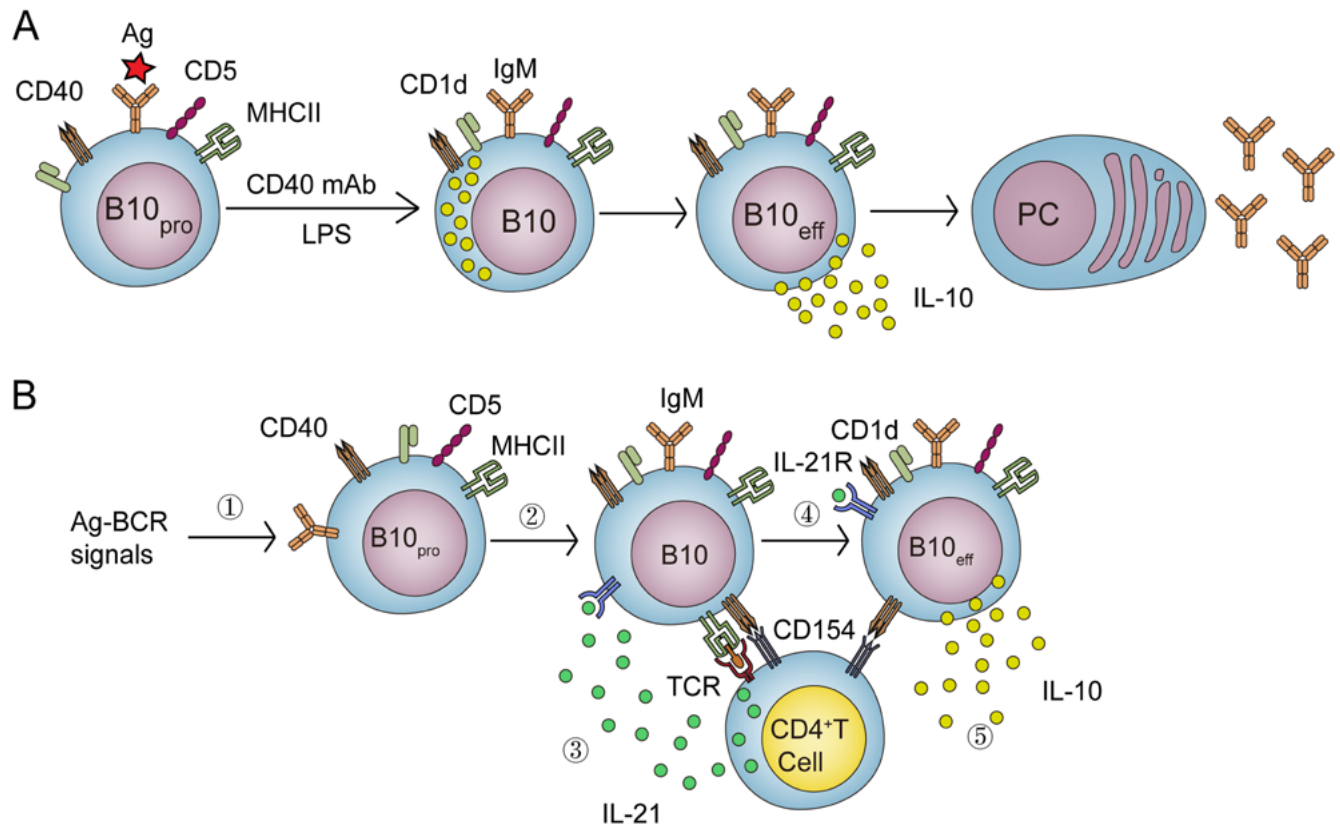
Low but detectable numbers of B10 cells are present in human peripheral blood, averaging around  $1.9 \pm 0.3 \times 10^3$  B10 cells/mL. These cells are also sporadically found in the spleen, tonsils and umbilical cord blood.<sup>17</sup> In human adult blood, B10 cells display elevated levels of CD19, as well as activation and memory markers such as CD27, CD48 and CD148. The expression of the memory markers, CD48 and CD148, suggests prior antigenic encounters *in vivo*.<sup>17</sup> Unlike immature or transitional B cells, B10 cells lack CD10 expression and have lower IgM levels. Levels of CD5, CD20, CD21, CD22, CD23, CD25, CD28, and CD40 expression are similar between B10 and typical B cells.<sup>17</sup> B10 cells are enriched within the CD24<sup>hi</sup> CD27<sup>+</sup> B cell subset, making up about 25% of all B cells in healthy individuals’ blood. This subsets has a tenfold higher proportion of B10 cells compared to the CD24<sup>lo</sup>CD27<sup>-</sup> subpopulation.<sup>17</sup> Additional studies have identified IL-10-producing human B cells with markers CD25<sup>hi</sup>CD27<sup>hi</sup>CD86<sup>hi</sup>CD1d<sup>hi</sup> B cells<sup>30</sup> and CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> B cells.<sup>31</sup>

## Development of B10 cell

Upon antigen-BCR binding, a cascade of appropriate signaling pathways is activated, leading B cells to release IL-10. These B cells are referred to as B10 cells (Fig. 1A).<sup>32</sup> A subpopulation of B cells, termed progenitor B10 cells (B10pro), receives the requisite signals for IL-10 production but has not yet attained full IL-10 secretory capability. Notably, B10pro cells lack definitive markers for identification. In the absence of specific stimulatory signals such as LPS or the CD40L/CD40 axis, these cells are incapable of IL-10 secretion. However, intracellular IL-10 production can be detected upon PIM stimulation. The transition from B10pro to fully functional B10 cells can be induced by a 48-h incubation with either CD40 monoclonal antibody (mAb) or LPS.<sup>19</sup> In human samples, B10pro cells constitute about 7% of peripheral B cells,<sup>33</sup> while in murine spleens, they generally represent 3–8% of the B cell population.<sup>19</sup> Importantly, studies indicate that B10pro cell frequencies remain stable in murine models during inflammatory and autoimmune conditions,<sup>16,19,22,24,27</sup> but show a marked upregulation in human samples.<sup>17</sup> This discrepancy suggests that the functional response of B10pro cells to inflammatory cues may be species-specific.

B10 cells that initiate IL-10 secretion are termed B10eff cells, although they are extremely rare *in vivo*. There is evidence that BCR-antigen interactions and BCR-mediated signaling are critical for normal B10 cell functionality.<sup>19</sup> For differentiation into IL-10-secreting B10eff cells, B10 cells require additional stimuli, potentially including CD40 signaling, IL-21, LPS or CpG.<sup>19,28</sup> Further studies indicate that B-lymphocyte stimulator (BlyS), also known as BAFF, significantly influences both the differentiation into B10 cells and their capacity to release IL-10.<sup>28</sup> Splenic B cells are co-cultured with NIH-3T3 cells expressing CD40L (CD154) and BAFF in the presence of IL-4 for 4 days. The old NIH-3T3-CD40L/BAFF cells are then replaced with fresh cells and IL-21 is added, this process is continued for another 5 days of co-culture, resulting in a 4 million fold increase in the number of B10 cells.<sup>28</sup> Some B10eff cells lose their IL-10-producing capability after transient *in vivo* expression, subsequently upregulating plasma cell-associated transcription factors like IRF4, PRDM1 and XBP1. This enables these B10eff cells to differentiate into plasma cells capable of generating polyreactive or autoreactive antibodies.<sup>26</sup> The regulatory implications of antibody production by B10eff cells remain unclear. Further research is being undertaken to understand the alternative fates of B10eff cells post-IL-10 secretion.

B10pro cells can develop normally in the absence of T cells, as evidenced by their presence in T cell-deficient nude mice.<sup>19</sup> Nevertheless, the interplay between B10 cells and CD4<sup>+</sup> T cells is essential for IL-10-mediated suppression of inflammation *in vivo*. Utilizing germline B-cell receptors (BCRs), antigen-specific B10 cells can capture antigens and present them to cognate CD4<sup>+</sup> T cells. This



**Fig. 1.** Mouse B10 cell development **A.** B cells acquire interleukin 10 (IL-10) activity. After B cells interact with specific antigens in vivo, appropriate levels of B-cell receptor (BCR) signaling induce a small proportion of B cells to become B10pro cells. IL-10 is not expressed by B10pro cells. However, when CD40 is ligated to B10pro cells ex vivo, the B10pro cells mature into fully functional B10 cells. Addition of PMA, ionomycin, and monensin (PIM) during the last 5 h of culture, mature B10pro cells and B10 cells were induced to express cytoplasmic IL-10. B10 cells that begin to secrete IL-10 become B10eff cells and can secrete IL-10 for 24–48 h in vivo. Upon termination of IL-10 transcription, a small number of B10eff cells differentiate into antibody-secreting plasma cells (PC) in vivo; **B.** The interaction between B10 cells and CD4<sup>+</sup> T cells. B cells capture antigens that trigger appropriate BCR signals (step 1) and promote the development of B10pro cells with IL-10 activity (step 2). B10pro presents antigenic peptides to antigen-specific T cells and induces T cell activation to produce IL-21 through homologous interaction and CD40/CD154 interaction (step 3). IL-21 binds to IL-21R in B10 cells (step 4) and promotes IL-10 production by B10 effector (B10eff) cells to negatively regulate antigen-specific T cell function (step 5). In this figure, mouse spleen B10 cells are shown as an example

B10pro – progenitor B10 cell; B10eff – B10 effector cell; PC – plasma cell.

contact is dependent on MHCII and CD40 molecules. Synchronized engagement between T cells and B10pro cells may expedite B10 cell maturation and promote T cell secretion of IL-21, which directly stimulates B10 cells to produce IL-10 in the local milieu.<sup>28</sup> The functions of proximal, homologously activated CD4<sup>+</sup> T cells and effector cells are dampened by antigen-specific B10 effector cells. Consequently, B10 cells lacking MHCII, CD40, IL-21R, or IL-10 are ineffective in mitigating autoimmune and inflammatory reactions. This crosstalk between B10 and T cells elucidates the antigen-specific regulatory roles of B10 cells in vivo.

## Function of receptors on B10 cell

Inflammation is a prerequisite for the in vivo proliferation and functionality of B10 cells. In both colitis and experimental autoimmune encephalomyelitis (EAE) models, B10 cells localize to the draining lymph nodes adjacent to inflamed tissues.<sup>27</sup> The ability of B10 cells to secrete IL-10 is determined by the inflammatory microenvironment; this was corroborated by the observation that splenectomy

in an EAE animal model did not impede B10 cell development or IL-10 secretion at these inflammatory sites.<sup>24</sup> The presence of various inflammatory mediators enhances B10 cell numbers and triggers IL-10 secretion. Similar dynamics can be observed in malignancies with an inflammatory component, such as colorectal cancer. Although the precise mechanism by which inflammatory signals drive B10 cell proliferation and IL-10 production remains unknown, it is likely linked to the high density of specific receptors on B10 cell surfaces. These receptors, in conjunction with inflammatory factors, may be responsible for B10 development and differentiation in tissue. Subsequently, we will discuss the relevance of selected B10 cell surface receptors and their functional implications.

## B-cell receptor

The BCR plays a role in inhibiting pro-inflammatory responses in both inflammation and cancer, as shown by studies in autoimmune disease models. Transgenic MD4 mice expressing a fixed BCR display a compromised ability



to prevent myelin oligodendrocyte glycoprotein (MOG)-induced autoimmune encephalomyelitis.<sup>34</sup> Results showed that B cell-derived IL-10 is pivotal for EAE amelioration. Under homeostatic conditions, MD4 mice exhibit a significantly reduced population of IL-10<sup>+</sup> B cells in the spleen, indicating the importance of BCR specificity and signaling in B10 genesis.<sup>19</sup> Additionally, the development of B10 cells is independent of MHCII, CD40, MyD88, or IL-10 receptor expressions on B-cells, underlining the critical role of BCR signaling in conferring IL-10 competence.<sup>19</sup>

B10 cells, localized in the spleen and peritoneal cavity, express a wide variety of BCR genes, majority of which are germline and devoid of somatic mutations.<sup>23,26</sup> This indicates that BCR specificity plays a crucial role on shaping B10 cell development and Ag-specific regulatory function.<sup>16,19,28</sup> B10 cell number can be significantly influenced by receptors or pathways that either positively or negatively affect BCR signaling, such as CD19, CD22 and CD40.<sup>23</sup> Interestingly, the number of IL-10-expressing B cells in both the peritoneum and the spleen diminishes when BCRs are robustly cross-linked with anti-IgM antibodies.<sup>19</sup> This suggests that a certain fraction of B cells may be primed to transition induced into B10pro and subsequently IL-10-competent B10 cells under low-affinity or persistent Ag-BCR signaling. Conversely, strong BCR signals could reroute B10pro cells towards alternative functional pathways.<sup>32</sup> Therefore, the specificity and strength of the BCR signal could enable B10 cells to swiftly react to both foreign and self-antigens, serving as an initial immunological defense while concurrently inhibiting autoantibody generation and safeguarding self-tissues.

## Toll-like receptors

Toll-like receptors (TLRs) are a group of proteins that can recognize specific molecular patterns on invading bacteria and viruses. Various TLRs are expressed on the surface of B10 cells, which regulate IL-10 secretion by the cells during inflammation. Through a MyD88-dependent mechanism, TLRs modulate B10 regulatory functions by enhancing IL-10 expression. This is evident when TLR2, TLR4 or TLR9 agonists stimulate splenic B10 cells *in vitro*.<sup>19,35,36</sup> In mice, LPS can induce the differentiation of spleen B10pro into B10 effector cells. Interestingly MyD88 is dispensable for B10 cell development *in vivo*.<sup>17</sup> Although various other signaling pathways can induce B10 cell activation, proliferation and effector function, BCR-associated signals appear to predominantly program B10 cells for IL-10 competence.

Intestinal IL-10-producing B cells are activated by resident enteric bacteria via TLR2, MyD88 and PI3K pathways,<sup>36</sup> subsequently dampening colonic T cell activation and maintaining mucosal homeostasis. Although the role of TLRs in tumor-associated B10 cell activation is unclear, it is possible that TLR signaling is activated by cancer-related byproducts. Exosomes from patients with esophageal

squamous cell carcinoma contain mRNAs that can activate TLR4 and MAPK signaling pathways, thereby promoting the differentiation of CD19<sup>+</sup> B cells into B10 cells in the peripheral blood.<sup>37</sup> In a separate study, Xiao et al. identified a novel pro-tumorigenic PD-1<sup>hi</sup> B10 cell subpopulation in human hepatocellular carcinoma (HCC).<sup>38</sup> The HCC-derived soluble factors activate TLR4 and elevate B-cell PD-1 expression. Significantly, these PD-1<sup>hi</sup> B cells acquire regulatory functions, suppressing tumor-specific T cell immunity in an IL-10-dependent manner. In addition, the TLR4-mediated BCL6 upregulation in the HCC micro-environment also resulted in the accumulation of PD-1<sup>hi</sup> B cells.<sup>38</sup> Moreover, intestinal extracts from mice with pancreatic cancer contain high concentration of TLR ligands that regulate B cell function by receptors binding, affecting disease progression.<sup>39,40</sup> Surface TLRs on B cells can regulate the cell's response to damage-related molecular patterns (DAMPs) produced during treatments like radiation and chemotherapy. Therefore, future research should investigate the effect of TLR function on tumor-associated B-cell behavior.<sup>41</sup> Animal models with conditional knock-downs of molecules like TLRs and MyD88 could offer valuable insights into B cell regulation in cancer.

## CD40

Although CD40 is not essential for IL-10 expression in B cells, its overexpression significantly increases the production of IL-10<sup>+</sup> B cells.<sup>19</sup> B10pro cells can mature to B10 cells *in vitro* by CD40 signal activation.<sup>19</sup> The role of CD40 signaling in specific B cell subsets within disease contexts remains unclear. In MC38 tumor-bearing mice lacking B-cell CD40, tumor growth was comparable to that in wild-type mice, indicating that CD40 on the surface of B cells is not necessary for tumor growth.<sup>42</sup> However, CD40L<sup>+</sup> EL4 thymoma cells co-cultured with wild-type B cells produced markedly more IL-10 compared to their interaction with CD40<sup>-/-</sup> B cells, indicating the effect may be model-specific.<sup>42,43</sup> In a mouse model of lupus, CD40 agonists induced B cell IL-10 secretion, thereby reducing inflammation and inhibiting disease progression. CD40 antibody treatment significantly improved survival rates in MRL/lpr lupus mice.<sup>44</sup> Additionally, it has been discovered that CD24<sup>+</sup>CD38<sup>+</sup> B cells engage with cancer cells via CD40 and CD40L interaction, leading to IL-10 and tumor growth factor beta (TGF- $\beta$ ) secretion, which in turn promotes tumor progression.<sup>45</sup> Given that both IL-10 production and B cell differentiation into antibody-producing cells are heavily influenced by CD40, further investigation into the molecular mechanisms governing B cell fate in response to CD40 signaling is imperative.

## Cytokine receptor

Even though B10 cells express IL-21R on their surface, incubating most B cells with IL-21 does not increase

IL-10 secretion.<sup>28</sup> The response of B cells to IL-21 varies according to their developmental stage and the presence of other costimulatory signals. Notably, IL-21 can induce apoptosis in addition to proliferation, survival, plasma cell differentiation, or isotype conversion of B cells.<sup>46,47</sup> However, IL-21R and IL-21 are required for B10 cell growth and the activation of IL-10-secreting B10 effector cell during the initiation of autoimmunity.<sup>28</sup> In the MOG-induced EAE mouse model, mice receiving IL-21R-deficient B cells showed enhanced MOG-TCR CD4<sup>+</sup> T cells proliferation and exacerbated disease symptoms compared to those receiving B cells from wild-type mice. This suggests that IL-21 can potentially suppress autoreactive T cells in vivo via a B cell-mediated mechanism.<sup>28</sup> Additionally, in tumorigenesis, IL-21 in tumor tissues can induce the generation of a population of CD38<sup>+</sup>CD1d<sup>+</sup>IgM<sup>+</sup>CD147<sup>+</sup> regulatory B cells, characterized by their granzyme B (GrB) secretion, thus designated GrB<sup>+</sup> B cells.<sup>48</sup> A significant subset of these GrB<sup>+</sup> B cells produce IL-10, highlighting that regulatory B cells in tumor milieu expressed both IL-10 and GrB.<sup>48</sup> Additionally, IL-21 activated B cells have demonstrated IL-10<sup>+</sup>CD25<sup>+</sup> regulatory phenotypes across various human tumor types.<sup>48</sup>

B10 cells, which express multiple inflammatory cytokine receptors including IL-21R, are directly regulated by pro-inflammatory cytokines.<sup>49</sup> This was evidenced in a mouse model of inflammatory arthritis, where elevated IL-6 and IL-1 $\beta$  levels fostered the expansion of B10 cells that limited the activity of autoreactive T cells.<sup>49</sup> In the context of oncogenesis, pro-inflammatory cytokines are critical in recruiting cells that support angiogenesis and tumor growth.<sup>50</sup> Both autocrine and paracrine effects of these

cytokines are observed in the tumor microenvironment. Animal models of pancreatic cancer showed that blocking IL-1 $\beta$  production in the tumor reduced IL-10-secreting CD1d<sup>hi</sup>CD5<sup>+</sup> B cells and amplified interferon gamma (INF- $\gamma$ ) and GrB-producing CD8<sup>+</sup> T cells, resulting in a stronger antitumor immune response and reduced tumor growth.<sup>51</sup> Furthermore, administration of a neutralizing IL-1 $\beta$  antibody to animals carrying pancreatic tumors produced similar results and, when synergized with  $\alpha$ -PD-1 therapy, significantly decreased the tumor burden.<sup>51</sup> This data suggest that the pro-tumorigenic activities of B10 cells, driven by inflammatory cytokines, could promote their own proliferation and IL-10 production, thus hindering the immune system's attack on the tumor.<sup>51,52</sup> As a result, cytokines can variably regulate B10 cell proliferation and IL-10 secretion in vivo.

## Mechanisms underlying B10 cell-mediated suppression of antitumor responses

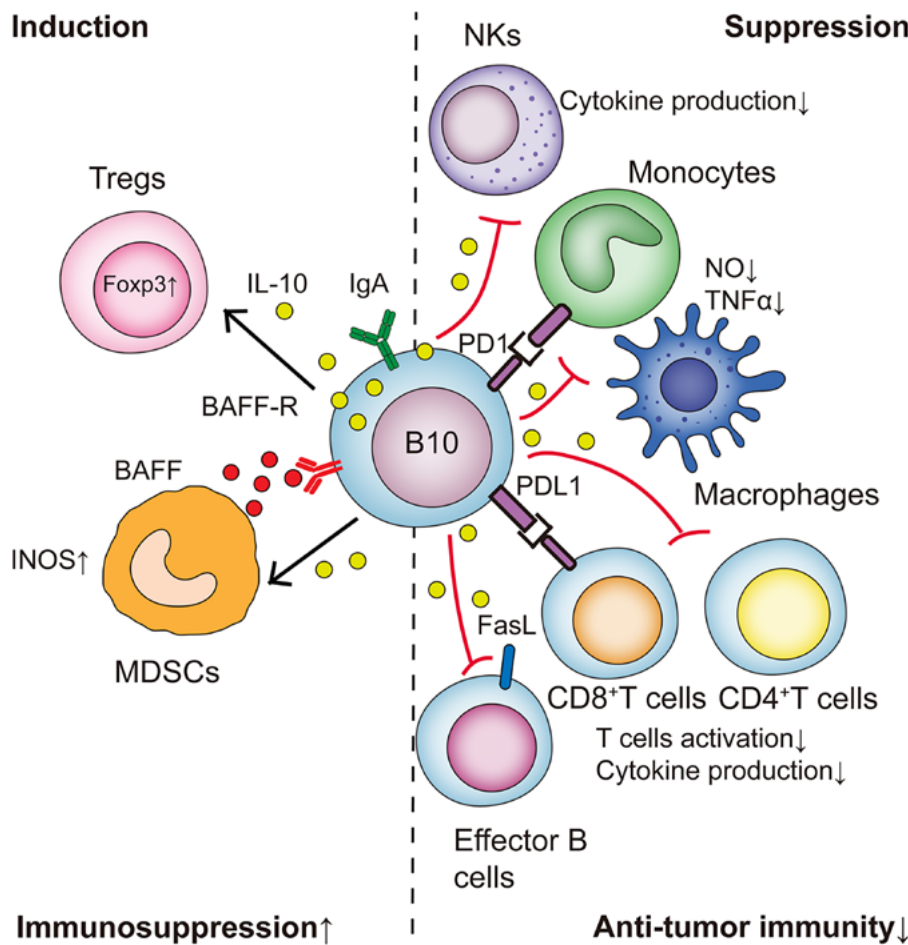
Cancer-related B10 cell immunoregulatory mechanisms have been gradually revealed. The equilibrium of all tumor infiltrating cells determines the nature of the relationship between immune cells, tumor cells and stromal cells in the tumor microenvironment. Multiple studies have recognized IL-10-producing B cells in tumor models; however, few have linked these properties to distinct B cell subsets (Table 2).<sup>38,53–61</sup>

B10 cells inhibit antitumor responses by interacting with different tumor-infiltrating immune cells. Moreover, B10 cells can participate in tumor progression by producing specific antibodies (Fig. 2).

**Table 2.** IL-10-producing B-cell phenotype and mechanism of action in human and mouse cancer

Phenotype	Species	Cancer type	Location	Mechanism	Reference
CD19 <sup>+</sup> CD1d <sup>hi</sup> CD5 <sup>+</sup>	mouse	non-Hodgkin lymphoma	spleen	IL-10-mediated suppression of macrophages activity	53
IgA <sup>+</sup> CD19 <sup>+</sup>	mouse	colorectal tumor	tumors	high expression of PD-L1, secretion of IL-10 and TGF- $\beta$ , mediated inhibition of CD8 <sup>+</sup> T cells, proliferation and activation	54
CD19 <sup>+</sup> CD5 <sup>+</sup> CD43 <sup>+</sup>	mouse	melanoma	tumors	IL-10 mediated suppression IFN- $\gamma$ and TNF- $\alpha$ by CD8 <sup>+</sup> T cells	55
CD5 <sup>hi</sup> CD24 <sup>-/+</sup> CD27 <sup>hi/+</sup> CD38 <sup>dim</sup>	human	HCC	tumors and PB	IL-10-mediated inhibition of CD8 <sup>+</sup> T cells	38
CD19 <sup>+</sup> CD1d <sup>hi</sup> CD5 <sup>+</sup>	human	cervical cancer and cervical intraepithelial neoplasia	PB	IL-10-mediated suppression perforin and GrB by CD8 <sup>+</sup> T cells	56
CD19 <sup>lo</sup> CD27 <sup>hi</sup> TIM-1 <sup>hi</sup>	human	colorectal cancer	tumors	IL-10-mediated the suppression of IFN- $\gamma$ and TNF- $\alpha$ secretion by T cells	57
CD19 <sup>+</sup> CD24 <sup>hi</sup> CD38 <sup>hi</sup>	human	GC	tumors and PB	IL-10-mediated the suppression of IFN- $\gamma$ and TNF- $\alpha$ secretion by CD4 <sup>+</sup> T cells	58
CD19 <sup>+</sup> CD27 <sup>+</sup> CD10 <sup>-</sup>	human	GC	tumors and PB	IL-10-mediated inhibition of IFN- $\gamma$ , TNF- $\alpha$ , and IL-17 secretion by CD8 <sup>+</sup> and CD4 <sup>+</sup> T cells	59
CD19 <sup>+</sup> IL-10 <sup>+</sup>	human	ovarian cancer	abdominal cavity	IL-10-mediated induction of T cells	60
CD19 <sup>+</sup> IL-10 <sup>+</sup>	human	TSCC	tumors	IL-10-mediated induction of T cells	61

GrB – granzyme B; HCC – hepatocellular carcinoma; HPV – human papillomavirus; TSCC – tongue squamous cell carcinoma; GC – gastric cancer; PB – peripheral blood; TSCC – tongue squamous cell carcinoma; IFN- $\gamma$  – interferon gamma; TNF- $\alpha$  – tumor necrosis factor alpha.



**Fig. 2.** Regulatory pathways and multifaceted interactions of B10 cells in the immune response. The dual role of B10 cells includes the induction and suppression mechanisms within the immune system. Induction pathways: 1. T cells: B10 cells participate in the proliferation and differentiation of T cells by secreting interleukin 10 (IL-10); 2. MDSCs: B10 cells and myeloid-derived suppressor cells (MDSCs) interact via BAFF and its receptor (BAFF-R), leading to increased expression of inducible nitric oxide synthase (iNOS). Suppression pathways: 1. Natural killer (NK) cells: Interactions with B10 cells lead to reduced cytokine production by NK cells, indicating a possible suppression or modulation of NK cell responses in the presence of IL-10; 2. Monocytes and macrophages: B cells induce the generation of B10 cells by binding to monocyte PD-1. B10 cells inhibit NO and tumor necrosis factor alpha (TNF- $\alpha$ ) production by monocytes and macrophages by secreting IL-10 and impair macrophage-mediated antitumor activity; 3. T cells: The activation of both CD8<sup>+</sup> cytotoxic T cells and CD4<sup>+</sup> helper T cells is notably diminished in the presence of B10 cells. The production of effector cytokines by these T cells is also reduced, highlighting the significant role of B10 cells in regulating T cell-mediated immune responses; 4. Effector B cells: Effector B cells can directly eliminate tumor cells by expressing FasL. B10 can inhibit the function of effector B cells through the secretion of IL-10

### Effect of innate immune cells activation

B10 cells play a crucial role in controlling the immune system's antitumor response. Rituximab is highly effective in treating non-lymphoma Hodgkin's and chronic lymphocytic leukemia (CLL), despite a high relapse rate.<sup>62</sup> A study has indicated that endogenous B cells, particularly CD1d<sup>hi</sup>CD5<sup>+</sup> B cells that produce IL-10 in CD20<sup>-/-</sup> mice, diminish the efficacy of CD20 mAb treatment.<sup>53</sup> In experiments, syngeneic Burkitt-like lymphoma-injected mice demonstrated a substantial increase in tumor size after receiving CD20<sup>-/-</sup>CD1d<sup>hi</sup>CD5<sup>+</sup>IL-10<sup>+</sup> B cells prior to anti-CD20 therapy, while IL-10-deficient CD1d<sup>lo</sup>CD5<sup>+</sup> B cells transfer had no impact.<sup>53</sup> Co-culture of CD1d<sup>hi</sup>CD5<sup>+</sup> B cells with macrophages revealed that CD1d<sup>hi</sup>CD5<sup>+</sup> B cells could inhibit macrophage production of nitric oxide and TNF- $\alpha$  in response to LPS, as well as downregulate the expression of MHC II molecules and co-stimulatory molecules, impairing macrophage-mediated antitumor activity. Interleukin 10 from CD1d<sup>hi</sup>CD5<sup>+</sup> B cells is essential to this inhibitory mechanism.<sup>53</sup> Natural killer (NK) cells, key components of the innate immune system's host defense, can directly lyse pathogen-infected or damaged cells and are actively involved in cancer immunology, particularly in directly lysing tumor cells.<sup>63,64</sup> Research using a B cell knockout mouse model with tumor showed that

the interaction between tumor cells and B cells via CD40L-CD40 can promote the production of a large amount of interleukin IL-10, which in turn hinders NK cells secretion of IFN- $\gamma$  and GrB, and subsequently impedes the antitumor function of NK cells.<sup>43</sup>

B10 cells not only impede the antitumor actions of innate immune cells but also promote their suppressive functions. For example, in the 4T1 breast cancer model, myeloid-derived suppressor cells (MDSCs) were observed to facilitate the proliferation of PD1<sup>-</sup>PDL1<sup>+</sup>CD19<sup>+</sup> IL-10-secreting B cells in the spleen, which in turn suppressed the expansion and IFN- $\gamma$  production of effector T cells.<sup>65</sup> This effect is actually reciprocal, as B10 cells can lead to immune escape in cervical cancer by enhancing the suppressive function of MDSCs. Furthermore, MDSCs can drive the differentiation of B cells into IL-10-rich B10 cells via BAFF/BAFF-R signaling, which, in turn, activates the STAT3 pathway in MDSCs, creating a self-amplifying loop. These activated MDSCs can suppress CD8<sup>+</sup> T cells through the release of inducible nitric oxide synthase (iNOS) and other immunoregulatory factors.<sup>66</sup>

### Suppression of effector T cell

B10 cells not only diminish the antitumor functions of innate immune cells, but also impair the antitumor

effects of adaptive immune cells. One crucial mechanism of B10 cells' impact on the antitumor response is direct suppression of effector T cell activity. In cervical cancer, CD19<sup>+</sup>CD5<sup>+</sup>CD1d<sup>+</sup> B cells produce IL-10, hindering CD8<sup>+</sup> T cells infiltration and subsequently reducing the fraction of cells producing perforin and GrB. The fraction of perforin- and GrB-producing CD8<sup>+</sup> T cells in tumor tissues can be restored with IL-10 blocking antibodies. B10 cells present in ascites drastically limit the number of IFN- $\gamma$ -secreting CD8<sup>+</sup> T cells in ovarian cancer.<sup>60</sup> Elevated levels of TIM-1-expressing CD19<sup>lo</sup>CD27<sup>hi</sup> B cells in colorectal cancer suppress IFN- $\gamma$  and TNF- $\alpha$  production by T cells via an IL-10-dependent mechanism.<sup>57</sup> Patients with gastric cancer (GC) exhibit a heightened frequency of IFN- $\gamma$ <sup>+</sup> and TNF- $\alpha$ <sup>+</sup>CD4<sup>+</sup> T cells in the peripheral blood, correlating with a scarcity of IL-10-producing CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup>B cells.<sup>59</sup> Co-culturing of CD27<sup>+</sup>CD10<sup>-</sup> B10 cells with autologous T cells from the peripheral blood or tumor tissues of patients markedly inhibits the capacity of CD4<sup>+</sup> T cells to express IFN- $\gamma$ , TNF- $\alpha$  and IL-17, as well as the ability of CD8<sup>+</sup> T cells to generate IFN- $\gamma$  and TNF- $\alpha$ . These findings are corroborated by animal studies,<sup>67</sup> with reports indicating that B1a cells (CD19<sup>+</sup>CD5<sup>+</sup>CD43<sup>+</sup>) use IL-10 to inhibit IFN- $\gamma$  and TNF- $\alpha$  production by tumor-infiltrating CD8<sup>+</sup> T cells, thereby exerting a negative regulation on tumor immunity in melanoma.<sup>55</sup> Furthermore, several studies demonstrate that B cells secreting IL-10 within tumors express PD-1 at high levels.<sup>37,38</sup> These PD-1<sup>hi</sup> B cells facilitate the depletion and dysfunction of CD8<sup>+</sup> T cells through IL-10, particularly upon activation via PD-L1 signaling in mice with hepatoma. This evidence underscores the role of PD-1/PD-L1 interactions in the suppressive functions of B10 cells within tumor environments.

## Induction of regulatory T cells

B10 cells not only modulate immune responses by controlling effector T cells but also promote the generation and/or maintenance of T cells, thereby contributing to an immunosuppressive microenvironment. Previous studies have postulated that B cells facilitate the conversion of resting CD4<sup>+</sup> T cells into T cells predominantly via TGF- $\beta$ , rather than IL-10.<sup>68</sup> However, evidence from several disease models suggests that a deficiency of B cell-specific IL-10 also correlates with reduced T cell numbers.<sup>33,69</sup> This implicates an involvement of IL-10 in T cell development, potentially explaining the observed correlation between increased T cell populations and accelerated cancer progression. B cells that produce IL-10 are present in the tumors in ovarian cancer and tongue squamous cell carcinoma.<sup>60,61</sup> These cells are not only inversely associated with disease prognosis but also positively correlated with the fraction of T cells (Foxp3<sup>+</sup>CD4<sup>+</sup> T cells) and inversely with the frequency of IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> T cells.<sup>60,61</sup> In vitro studies involving co-culture of tumor-derived CD19<sup>+</sup>IL-10<sup>+</sup> B cells with autologous CD4<sup>+</sup>CD25<sup>-</sup> T cells from patients with tongue

squamous cell carcinoma have shown that these T cells can differentiate into T cells through an IL-10 dependent mechanism provided by CD19<sup>+</sup>IL-10<sup>+</sup> B cells.<sup>61</sup> These results reveal that B10 cells may facilitate T cell development within the tumor microenvironment. Nevertheless, further investigation is needed to fully understand the mechanism behind this T cell induction by B10 cells in the tumor context. Conversely, regulatory T cells may also enhance the proliferation and differentiation of B10 cells in tumors. A newly identified T cell subset, follicular regulatory T cells (Tfr), has been shown to induce IL-10 production in B cells within the context of breast cancer<sup>70</sup>

## Other tumor-infiltrating targets of B10 cells

B cells have both antitumor and pro-tumor effects. Tao et al. have shown that effector B cells from tumor-draining lymph nodes (TDLNs) express Fas ligand (FasL) in vitro, which can directly eliminate 4T1 tumor cells. This suggests that the adoptive transfer of TDLN B cells could enhance their therapeutic effect. However, IL-10-producing B cells within TDLNs may suppress the antitumor functions of these effector B cells.<sup>71</sup> Remarkably, depletion of IL-10 from B-cell adoptive transfers significantly enhances CTL activity within the recipient's peripheral blood mononuclear cells (PBMCs) and splenic cells, as well as B-cell function.<sup>71</sup>

B cells may also facilitate tumor growth by producing specific antibodies, yet the role of B10 cells in tumor suppression via this mechanism remains to be verified. The current consensus posits that antibody-secreting B cells, especially those producing IgA, regulate T cell-mediated antitumor immunity in 1 of 2 major ways: either through the expression of immune checkpoint molecules like PDL1 or by releasing inhibitory cytokines such as IL-10. These mechanisms, which can occur via direct contact or indirectly, ultimately contribute to the diminished effectiveness of T cells in eliciting an antitumor immune response.<sup>54,72</sup> The presence of IgA<sup>+</sup>PD-L1<sup>+</sup>IL-10<sup>+</sup> plasma cells in prostate cancer tissues has been shown to contribute to chemotherapy resistance.<sup>72</sup> The underlying mechanism involves chemotherapeutic agents like oxaliplatin promoting plasma cell migration into tumors, where these cells express PDL1 and produce TGF- $\beta$  and IL-10, thereby dampening CD8<sup>+</sup> T cell activation. This means that IgA expression alone does not account for the immune suppression observed. The ablation of IgA<sup>+</sup>PD-L1<sup>+</sup>IL-10<sup>+</sup> plasma cells could increase the activity of CD8<sup>+</sup>T cells and restore the oxaliplatin-mediated regression of tumors. In a mouse model of colorectal cancer, tumor-infiltrating IgA<sup>+</sup> B cells release a high number of immunoregulatory molecules (PD-L1, IL-10 and TGF- $\beta$ ), which restrain CD8<sup>+</sup> T cells limiting the proliferation.<sup>54</sup> Reducing the presence of IgA<sup>+</sup> B cells in colorectal tumors correlates with extended patient survival. These findings bolster the hypothesis that



antibody-secreting B cells are integral in promoting tumor growth by inhibiting cytotoxic T cell responses.

Recent research largely supports the notion that B10 cells facilitate tumor progression and metastasis. However, B10 cells may also serve a protective role in oncogenesis. For example, Melcher et al. found that B cell-derived IL-10 can regulate chronic intestinal inflammation but not colitis-associated colorectal cancer (CAC). Upon differentiation into plasma cells, B cells cease IL-10 production and begin the secretion of IgA that modulates the gut microbiota composition and potentially guards against CAC development.<sup>73</sup> Similarly, patients infected with *Helicobacter pylori* exhibiting elevated high levels of IL-10-secreting B cells are less prone to developing gastric cancer. It is proposed that B cells may downregulate tissue-damaging proinflammatory responses triggered by bacterial infection, thereby creating an environment that hinders tumor progression.<sup>74</sup> These observations add another level of complexity to the biology of B10 cells in cancer.

## Discussion

### Targeting B10 cells

There is growing evidence that B10 cells play an immunosuppressive role in cancer, even though their suppression is typically beneficial in autoimmune disease.<sup>75,76</sup> The benefits of entirely eliminating B cells for cancer patients remain uncertain. While studies on certain cancers, such as colon cancer and melanoma, have shown that the widespread reduction of CD20<sup>+</sup> cells using anti-CD20 antibodies can be beneficial, other studies report no benefit or even harmful effects.<sup>77–82</sup> This discrepancy may reflect the dual regulatory roles played by B cells in tumor development. Presently, several therapies focus on the selective reduction of B10 cell populations.<sup>83,84</sup> Small molecule inhibitors targeting B10 cell activity may also prove beneficial.<sup>84</sup> In cancer mouse models, MAP kinase pathway inhibitor and BTK inhibitor selectively suppress IL-10 production and the proliferation of CD1d<sup>hi</sup>CD5<sup>+</sup>, TIM-1<sup>+</sup> and CD21<sup>+</sup>CD23<sup>+</sup>CD24<sup>+</sup> B cell subsets without impacting other B cell subsets.<sup>52,83</sup> Total glucoside of paeony (TGP) demonstrates antitumor effects in rat HCC by reducing the number of B10 cells in the spleen of treated rats.<sup>84</sup> Lipoxin A4, also known as LXA4, is an anti-inflammatory lipid mediator derived from arachidonic acid that can reshape the tumors immune microenvironment and confer antitumor properties.<sup>85</sup> Lipoxin A4 inhibited B10 cell induction in tumor-bearing mice, which decreased T cell numbers in both lymph node draining and tumor tissue while enhancing cytotoxic T cell activity.<sup>85</sup> Intriguingly, LXA4 can specifically suppress B10 cell generation without interfering with the proliferation, differentiation, or germinal center formation of conventional B cells.<sup>85</sup> Furthermore, studies have shown that targeting IL-10R1 selectively to B cells

with CD19 single chain antibody can decrease the number of B10 cells and T cells, augment the activity of cytotoxic CD8<sup>+</sup> T cells, and inhibit the progression of HCC.<sup>86</sup> The oncogenic function of CXCL13 across diverse desmoplastic mouse tumor models has been elucidated. Shen et al. found that within the tumor microenvironment (TME), tumor-associated fibroblasts secrete CXCL13, which is pivotal for the recruitment and subsequent differentiation of B cells into IL-10<sup>+</sup>CD1d<sup>+</sup>CD5<sup>+</sup>CD138<sup>+</sup>CD19<sup>+</sup> cells. The inhibition of CXCL13 has been shown to curtail tumor progression.<sup>87</sup> These findings underscore the potential of B10 cell blockade as an effective approach to impede cancer metastasis. Further investigations are imperative to devise a cancer treatment strategy that targets B10 cells.

The exploitation of immune checkpoints, specifically the PD-1 and PD-L1 pathways, by tumors facilitates immune evasion by attenuating the activation of immune cells.<sup>88</sup> The presence of PD-1 is notable on a spectrum of activated immune cells – ranging from monocytes to T cells – and is indicative of the receptor's regulatory role. PD-L1, correspondingly upregulated within the tumor milieu on both tumor and presenting cells, becomes a focal point for therapeutic intervention.<sup>89</sup> Disruption of the PD-1–PD-L1 interaction has yielded significant tumor regression and prolonged survival in both clinical and preclinical settings.<sup>88</sup> It has been previously observed that B cells with concurrent PD-1 or PD-L1 and IL-10 expression attenuate CD4<sup>+</sup> and CD8<sup>+</sup> T cell functions following PD-1/PD-L1 signal activation, which implies a potential inducement of IL-10 by PD-1/PD-L1 pathways.<sup>37,38,65,72,75,90,91</sup> This is exemplified by IL-10<sup>+</sup>PD-L1<sup>+</sup> B cells in mouse pancreatic cancer models, where they diminish T cell viability – an effect reversible by PD-L1 inhibition.<sup>90</sup> Additionally, IgA<sup>+</sup>PD-L1<sup>+</sup> B cells, which are capable of secreting IL-10, were discovered in models of both liver and prostate cancer. These B cells were found to be responsible for promoting chemoresistance, a process that can be reversed by PD-L1 targeting.<sup>72,91</sup> These insights reinforce the influential role of B cells in the milieu of PD-1/PD-L1-targeted therapies and suggest that targeting PD-1 and PD-L1 on B cells harbors therapeutic potential. There is an ongoing need to unravel the intricacies of immune checkpoint therapy-driven antitumor responses to refine immunotherapeutic strategies.

### Limitations

B10 cell biology has advanced significantly in recent years, but the specifics of their development, phenotypic, functional, and suppressive mechanisms remain unanswered. To begin, B10 cells are found beyond the spleen, in the abdominal cavity, lymph nodes, and other organs. However, it is unclear whether IL-10 production mechanisms are consistent across these varied locations. The second issue is that B10 cells are known for IL-10 secretion, but it is uncertain if they exert regulatory role through other cell surface molecules, such as co-stimulatory molecules.


Furthermore, the role of B10 cells in the tumor micro-environment is crucial to understand, specifically, how they facilitate tumor growth while dampening inflammation. Unraveling this could improve antitumor immunity by targeting B10 cells in cancer. The complex processes of B10 cell generation and activation, especially in cancer progression, warrant in-depth investigation.

## Conclusions

B10 cells are a subset of B cells that regulate immune responses, such as inflammation and tumor suppression. B10 cells perform their functions by interacting with other immune cells and secreting cytokines. The proliferation and function of B10 cells rely on the existence of cancer-induced inflammation. B10 cells within the TME hinder antitumor immunity by suppressing effector T and B cells, inducing regulatory T cells, and impairing other tumor-infiltrating immune cells including NK cells and macrophages. B10-generated antibodies are also implicated in tumor immune escape mechanisms (Fig. 2). Moving forward, research in B10-based immunotherapies should prioritize identifying the surface markers expressed on B10 cells.

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