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B Cell Function in the Tumor Microenvironment

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Abstract

The tumor microenvironment (TME) is a heterogeneous, complex organization composed of tumor, stroma, and endothelial cells that is characterized by cross talk between tumor and innate and adaptive immune cells. Over the last decade, it has become increasingly clear that the immune cells in the TME play a critical role in controlling or promoting tumor growth. The function of T lymphocytes in this process has been well characterized. On the other hand, the function of B lymphocytes is less clear, although recent data from our group and others have strongly indicated a critical role for B cells in antitumor immunity. There are, however, a multitude of populations of B cells found within the TME, ranging from naive B cells all the way to terminally differentiated plasma cells and memory B cells. Here, we characterize the role of B cells in the TME in both animal models and patients, with an emphasis on dissecting how B cell heterogeneity contributes to the immune response to cancer.

1. INTRODUCTION

The tumor microenvironment (TME) is a complex structure composed of tumor cells, stromal and endothelial cells, and immune cells, with dynamic interactions and cross talk between these cells. Over the last decade, a number of studies have shown that adaptive immune cells, specifically T lymphocytes, are critical in the antitumor immune response (1–3). Decrease of effector function of T cells, especially $CD8^+$ T cells in the presence of antigen, a process termed exhaustion, leads to a dysfunctional immune response in the TME (4). These exhausted T cells express specific proteins, termed checkpoints, that are associated with this dysfunctional program. $CD4^+$ T cells have greater heterogeneity and mixed functions in the TME. T helper type 1 (Th1) effector cells that generate IFN-γ and TNF are intermixed with Th17 cells that express IL-17A, IL-17F, and GM-CSF (granulocyte-macrophage colony-stimulating factor). In contrast, T cells that express FoxP3, termed regulatory T cells (Tregs), suppress antitumor immune responses (5). This review does not focus on the function of T cells and exhaustion in the TME; individuals interested in this critical area of tumor immunology should reference the large number of reviews of the functions of T cells in the TME recently published (6–8).

B cells are a second population of adaptive immune cells found in the TME. The functions of B cells are complex and challenging, as transcription factors defining subsets of B cells have not been described. B cells are typically classified according to their expression of specific surface proteins, which may not adequately reflect the heterogeneity of these cells in the TME. Over almost two decades, a number of studies have suggested that B cells have protumorigenic properties. However, in the last five years, multiple studies, including a number from our group, have demonstrated an association between the presence of B cells in the TME and improved clinical outcome. Here, we evaluate the different roles of B cells in the TME and discuss critical new areas for research into the functions of these cells in antitumor immunity.

1.1. Overview of Normal B Cell Development and Biology

B cell development in the bone marrow is characterized in **Figure 1**. Before exiting the bone marrow, B cells undergo negative selection. A large portion of these immature B cells are self-reactive and undergo receptor editing or clonal deletion by apoptosis or anergy (9–11) critical to the maintenance of self-tolerance (12–14). Self-reactive cells that can successfully edit themselves and those that are weakly or non-self-reactive mature in the spleen into transitional B cells. These cells now express a rearranged B cell receptor (BCR) and B220 (15). Within the spleen, B cells develop through a second round of negative selection as they pass through the transitional zone stage (T1 and T2) (16). A small portion of B cells will remain in the spleen as noncirculating marginal zone B cells. The remainder become follicular B cells, which circulate among splenic follicles, lymph nodes and bone marrow (17) (**Figure 1**; **Table 1**).

Both marginal zone and follicular B cells are part of the extrafollicular response to antigen. Marginal zone B cells are the first cells to respond to foreign antigen and are notable for their rapid response to antigens. Their location in the splenic marginal zone gives them early access to circulating antigens. Here they can respond to T cell–independent antigens, after which they proliferate into plasmablasts and secrete immunoglobulin. The differentiation of naive B cells into extrafollicular B cells tends to generate short-lived, antibody-secreting cells of lower affinity, although recent data in animal models suggest that the extrafollicular B cells can undergo affinity maturation and generate memory B cells and long-lived antibody-secreting cells even in the absence of T cell help (18). Circulating follicular B cells can differentiate into plasmablasts and plasma cells (PCs) after encountering T cell–dependent antigens and do so more slowly than marginal zone B cells. PCs from these two types of encounters are short-lived and in most instances

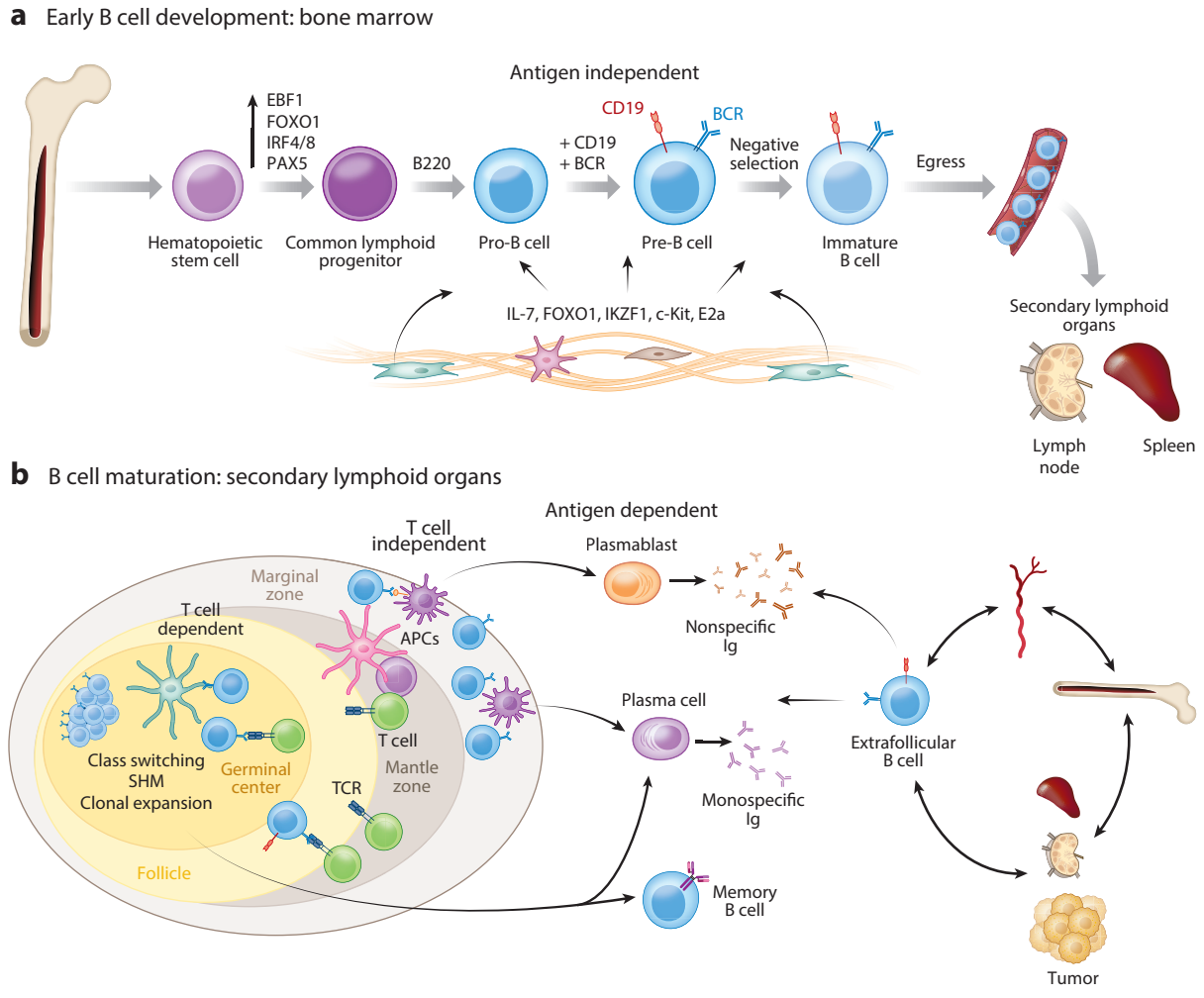

Figure 1

B cell development and maturation. (*a*) B cell development begins in the bone marrow, where hematopoietic stem cells encounter factors such as EBF1, FOXO1, and PAX5, resulting in lineage commitment to produce common lymphoid progenitors. Through the acquisition of B220, CD19, and a BCR, pro-B cells develop into pre-B cells and finally immature B cells. Stromal support is needed to drive this process, as it is the source of key B cell factors such as IL-7 and is critical for the induction of the B cell–specific transcription factors FOXO1 and IKZF1, among others. Prior to egress from the bone marrow, B cells undergo negative selection to eliminate any self-reactive clones. (*b*) B cell maturation occurs in secondary lymphoid organs throughout the body, where antigen exposure via both T cell–dependent and T cell–independent processes shapes the B cell repertoire. Germinal center follicles are the primary site where somatic hypermutation and class switching occur, with proliferation and differentiation eventually leading to the generation of plasma cells capable of producing highly specific antibodies, or long-lived memory B cells. In the marginal zone, developing B cells can undergo antigen presentation in a T cell–independent fashion, resulting in plasmablast generation. Circulating extrafollicular B cells can differentiate into plasmablasts, plasma cells, and memory B cells and traffic between secondary lymphoid organs and bone marrow. Abbreviations: APC, antigen-presenting cell; BCR, B cell receptor; Ig, immunoglobulin; SHM, somatic hypermutation; TCR, T cell receptor.

have not undergone affinity maturation or somatic hypermutation. These B cells are important for the initial but, in most instances, not sustained response to antigens (15). Follicular B cells that have encountered antigen and received T cell help may have an alternative fate through the germinal center reaction, when they mature into PCs that secrete high-affinity antibodies. Through

Table 1 B cell subsets

Type of B cell	Function
Transitional (T1, T2, and T3)	■ Precursor to full B cell maturation (dependent on BCR engagement and BTK activation) ■ Can secrete IL-10 to suppress T cell proliferation and proinflammatory cytokine release
Follicular (B-2)	■ Participate in T cell–dependent activation and antibody response ■ Undergo somatic hypermutation, class switching, and affinity maturation to generate diverse BCR repertoire ■ Can differentiate into plasmablasts or plasma cells for immunoglobulin production ■ Primary source of memory B cell production
Marginal zone (B-2)	■ Involved in T cell–independent antigen presentation and activation ■ Differentiate into plasmablasts for nonspecific immunoglobulin production
B-1	■ Associated more with humoral immunity and respond to bacterial and polysaccharide antigens; some role in vaccine response ■ Predominately secrete IgM ■ Tissue-resident; also found in peritoneum and pleura; important for mucosal innate immunity
Extrafollicular	■ Circulating, maturing B cell population ■ Can undergo T cell–dependent and –independent antigen presentation and activation ■ Differentiate into plasmablasts, plasma cells, and memory B cells
Memory	■ Long-lived, with high-affinity BCR to respond rapidly to antigen rechallenge and drive secondary immune response ■ Can serve as antigen-presenting cells for T cell activation
Plasmablasts	■ Short-lived; produce nonspecific immunoglobulins ■ Involved in initial response to antigen though not sustained
Plasma cell	■ Terminally differentiated B cells; produce highly specific antibodies
Regulatory (i.e., B10)	■ Produce cytokines (i.e., IL-10 and IL-35) to inhibit effector T cell function ■ Promote immunosuppressive environment, including T cell differentiation to Tregs

Abbreviations: BCR, B cell receptor; BTK, Bruton tyrosine kinase; Treg, regulatory T cell.

the germinal center reaction, B cells can differentiate into PCs or switched memory B cells, which retain a high-affinity BCR, and can respond rapidly to rechallenge with antigen (15). PCs are the final effector cell in the B cell differentiation pathway and result from marginal zone or follicular B cell activation or from the germinal center reaction of activation of memory B cells. The expression of BCL6/BACH2/PAX5 is critical to maintain the B cell phenotype and promote B cell proliferation and somatic hypermutation, while the expression of IRF4/PRDM1/XBP1 blunts B cell proliferation and promotes class switch recombination and differentiation of these cells to PCs, which are required for productive secretion of immunoglobulins, the effector molecules of PCs (19, 20).

2. REGULATION OF B CELL RESPONSE

B cells are activated when the BCR is bound by either soluble or membrane-bound antigens. The latter have a lower threshold for activation than their soluble counterparts (21). This results in activation of immunoreceptor tyrosine-based activation motifs due to phosphorylation by Lyn and Syk, which leads to the activation of PI3K (phosphatidylinositol 3-kinase), PLCγ, Vav, and Map kinase proteins, which results in proliferation and ultimately antibody production (22). A major negative regulator of the B cell response is the molecule CD22, which through its interaction with CD22L is crucial for maintaining self-tolerance (20).

2.1. B Cell Homing to the Tumor Microenvironment

How immune cells gain access to the TME has been an area of intense research by a number of groups, including ours, demonstrating the therapeutic relevance of optimizing the peri- and intratumoral immune infiltrate (23–25). A number of studies have now shown that ectopic lymphoid neogenesis occurs within the TME that is made up of immune cells with interactions similar to those that occur in secondary lymphoid organs (SLOs) (26–34). Previous work demonstrated that B cells preferentially localize in these aggregates and their formation is correlated with antitumor control (35). Tertiary lymphoid structures (TLSs) are associated with both a tolerogenic environment and an enhanced antitumor immune effector response. The process of TLS generation has previously been extensively reviewed (36, 37); here we focus on the processes governing B cell recruitment, interactions within TLSs, and function in the intratumoral environment.

2.2. Tertiary Lymphoid Structures in the Tumor Microenvironment

Review of intratumoral specimens shows the full progression of lymphoid development from an early TLS to a primary follicle TLS and finally a mature TLS characterized by a B cell–predominant germinal center surrounded by a T cell aggregate (31). While TLSs have been appreciated in both the peritumoral and intratumoral environments, their location in the latter is associated with improved survival (38). Further, TLSs are found in a majority of pretreated tumor samples in patients that have B cells producing tumor-specific antibodies (39). The interaction of follicular dendritic cells (FDCs) with B cells is critical to the generation of germinal centers and maturation of SLOs. In tumors, B cells colocalize with $CD21^+$ FDCs in TLSs near $MECA79^+$ high endothelial venules, a similar arrangement found in lymph nodes (31). Suggesting a role in the antitumor immune response, T cell activation is greater in TLSs as compared to outside of these structures (see **Figure 2**).

2.3. Chemokines and Tertiary Lymphoid Structure Development

The chemokine CXCL13, produced by FDCs, is critical to SLO development (40, 41), and the receptor for this chemokine, CXCR5, is important for B cell localization. In the TME, CXCL13 is essential for B cell recruitment but is not produced solely by FDCs. Gu-Trantien et al. (42) showed that in breast cancer, CXCL13 production is dependent on the presence of T follicular helper (Tfh) cells termed TfhX13 cells. They observed that B cells clustered around these Tfh cells in breast tumors, whereas they clustered around FDCs in tonsillar control samples. The TfhX13 cells were important for B cell recruitment into the peritumoral area and precursor TLS sites, and ultimately for germinal center formation. This finding is consistent with studies in murine models where SLOs can develop in the absence of FDCs (43, 44).

Interestingly, CXCL13 generation by TfhX13 cells has been linked to IL-2 deprivation and TGF-β production. This mechanism has parallels to the development of tissue-resident $CD8^+$ T cells where TGF-β signaling leads to the expression of CD103 and promotes tissue residency (45). Increased secretion of CXCL13, B cell recruitment, and TLS formation strongly correlated with the presence of $CD103^+CXCL13^+CD8^+$ tumor-infiltrating lymphocytes (TILs). This was seen across tumor types, including lung, ovarian, breast, and uterine tumors, and correlated with improved survival (46).

TLSs within the TME are correlated with improved clinical outcome in patients with cancer. More interestingly, these structures may be critical in the response to cytotoxic chemotherapy, small-molecule inhibitor therapy, and immune checkpoint inhibitor therapy and cancer recurrence (33, 47, 48). B cells are particularly important to redirect the anticancer immune response,

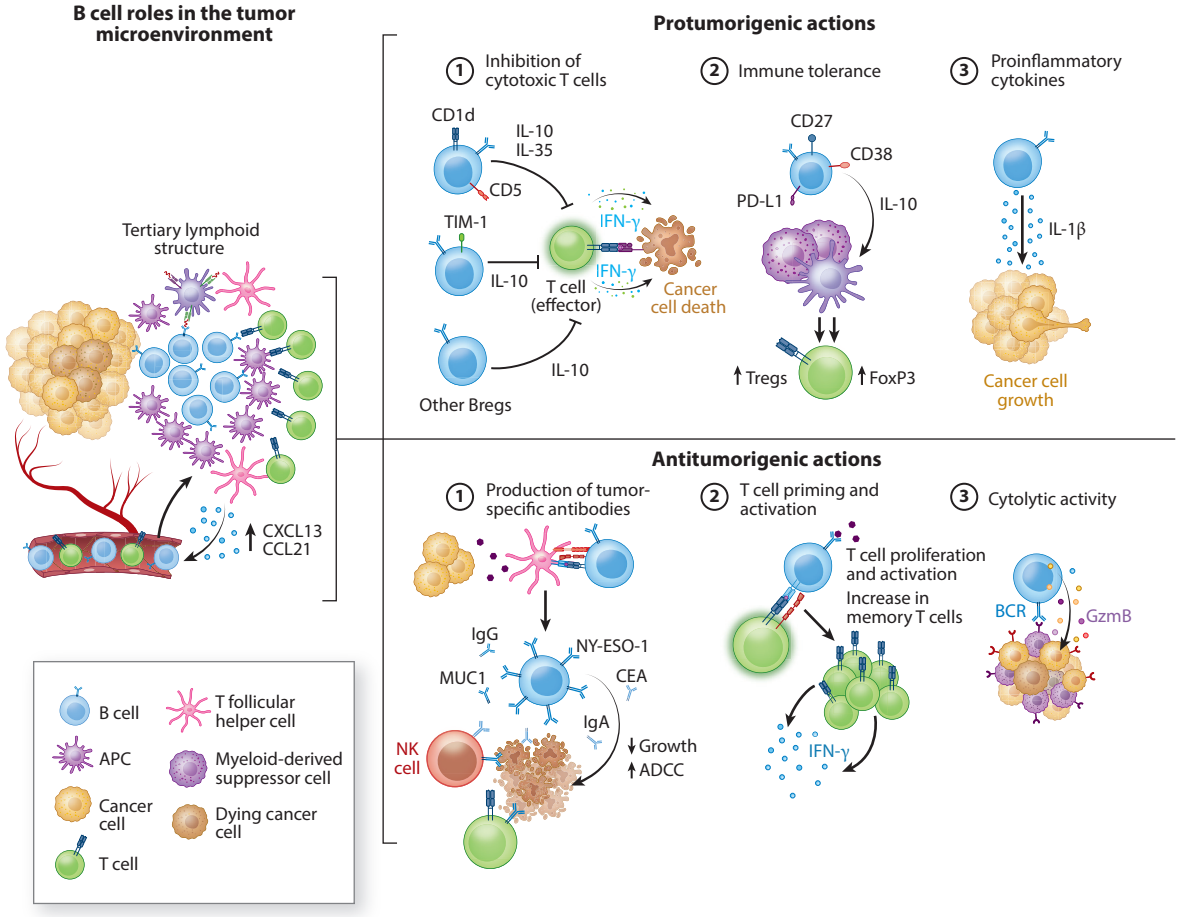

Figure 2

B cell actions in the TME. B cells are recruited to the TME by chemokine gradients, with CXCL13 and CCL21 featuring prominently. Once there they localize to tertiary lymphoid structures, which resemble germinal centers, where B cells interact with APCs and peripheral T cells. B cells within the TME may have both pro- and antitumorigenic roles. (*Top*) B cells can promote tumor growth by the following mechanisms: (①) direct inhibition of T cells through the production of IL-10 by $CD1d^+/CD5^+$ and $TIM\text{-}1^+$ Bregs, in addition to other cells that generate IL-10; (②) promotion of immune tolerance by $PD\text{-}L1^+$ B cells that act on innate immune cells to lead to a microenvironment favoring Treg development; and (③) generation of proinflammatory cytokines like IL-1β that can directly drive cancer cell growth. (*Bottom*) At the same, emerging data have shown that B cells can also serve an antitumorigenic role. (①) Following antigen presentation, B cells can secrete tumor-specific antibodies that lead to decreased growth and ADCC mediated by NK cells and myeloid cells. (②) B cells can serve as APCs themselves to prime T cells and lead to T cell activation and clonal expansion and memory T cell formation, culminating in T cell–mediated processes, like IFN-γ production, to limit tumor growth. (③) B cells can directly kill tumor cells when BCRs recognize tumor cell antigens, leading to granzyme B production. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; APC, antigen-presenting cell; BCR, B cell receptor; Breg, regulatory B cell; NK, natural killer; TIM, T cell immunoglobulin domain and mucin domain; TME, tumor microenvironment; Treg, regulatory T cell.

as they may counteract the immunosuppressive environment. In tumors that have a higher percentage of B cells, there are increased numbers of central memory and activated $CD4^+$ T cells and decreased Tregs (29), as well as enhanced generation of $CD8^+$ T cells and increased expression of IFN-γ (49). This is, however, not a straightforward finding, as the presence of exhausted B cells, characterized by increased expression of CD21 and CD27, was associated with tumor progression

and enhanced expression of Tregs, highlighting the complexity of the B cell–T cell interaction in the TME.

3. B CELL HETEROGENEITY IN THE TUMOR MICROENVIRONMENT

There is significant heterogeneity in both the surface immunophenotype and function of the B cell populations present in the TME (**Figure 2**). Using mass cytometry to interrogate a large number of surface proteins, investigators demonstrated the presence of naive B cells, memory B cells, activated memory B cells, and plasmablasts (31). Interestingly, B cells generating IL-10, IL-35, or TGF-β have not been found in breast tumors prior to therapy (50). Compared to the peripheral blood, the TME has increased switched memory B cells and antibody-secreting B cells. There are differences in surface marker expression in B cells found within the tumor compared to those in the peripheral blood, with upregulation of costimulatory proteins such as CD86 and decreased expression of CD23. This is consistent with increased activation of B cells in the TME. CD23 is downregulated in mature B cells that have undergone class switching and somatic hypermutation, as it serves as a negative regulator of B cell activity (51).

The heterogeneity of tumor-infiltrating B cells (TIL-B cells) is similar in other cancers, including head-and-neck squamous cell carcinoma and gastroesophageal adenocarcinoma, with increased numbers of activated and memory B cells (34). Memory B cells are increased in tumors compared to the peripheral blood, making up 34% compared to 14%, respectively, of B cells. This is independent of tumor grade. Interestingly, increased numbers of memory B cells, $CXCR3^+$ cells, and germinal center–like B cells are found in the TME in patients that have responded to immune checkpoint inhibition (ICI) therapy (31).

Pancreatic ductal adenocarcinoma tumors that have TLSs are enriched in memory B cells as compared to tumors that do not (52). TLSs found in the TME had fewer IgD^+ and IgM^+/IgD^+ naive B cells compared to the peripheral blood, suggesting that class switching occurs in the TME. There was an increase in IgG1 and somatic hypermutation of B cells in the TME in tumors with TLSs. Thus, there may be an ongoing process in the TME of affinity maturation and clonal selection that could markedly enhance the antitumor immune response. The type of immunoglobulin generated is tumor dependent. IgG is the largest fraction in thyroid, testicular, and cutaneous tumors, whereas IgA constitutes the largest fraction of immunoglobulins in kidney, ovarian, pancreatic, and colorectal cancers (53, 54).

In addition to their ability to generate cytokines and differentiate into plasmablasts, B cells can function as antigen-presenting cells (APCs) to present antigen to T cells (**Figure 2**). B cells that function as APCs often have increased expression of costimulatory proteins critical for T cell activation. In patients with ovarian cancer, B cells with increased expression of CD80, CD86, and MHC-II proteins are found juxtaposed with $CD8^+$ T cells (55). Our group and others have demonstrated a clear association between improved prognosis and the presence of B cells and T cells using genomic approaches (23, 24) or cellular immunology assays (25). This strongly suggests that B cells are critical to the robustness and magnitude of the T cell response in cancer.

4. PROTUMORIGENIC FUNCTION OF B CELLS

The initial data indicating a protumorigenic role for B lymphocytes come from studies involving tumor growth in mice with genetic deficiencies in B lymphocytes or after the depletion of these cells using monoclonal antibodies. The growth of multiple murine tumor cell lines was decreased or eliminated in animals lacking B cells, such as μMt or $Jh^{-/-}$ mice (56, 57). In these models, the absence of B cells led to enhanced effector function of T and/or natural killer (NK) cells,

Table 2 Regulatory B cells identified in murine tumors

Cancer type	Model	Surface markers	Effector functions	Reference(s)
Breast cancer	4T1 (triple negative)	$CD19^{+}B220^{+}CD25^{+}CD69^{hi}$	TGF-β	94
	EMT-6	PD-$L1^{hi}CD86^{hi}IAd^{hi}CD62L^{hi}LAP^{+}CD44^{lo}$	TGF-β, PD-L1	101
Hepatocellular carcinoma	NASH (high-fat diet induced)	$CD19^{+}B220^{lo}CD138^{+}IgA^{+}$	PD-L1	155
Non-Hodgkin lymphoma	BL3750 (Burkitt-like lymphoma)	$CD19^{+}CD1d^{hi}CD5^{+}$	IL-10	156
Pancreatic ductal adenocarcinoma	LSL-$K\text{-}ras^{G12D}$, Pdx1-Cre, and p48-Cre	$CD19^{+}CD1d^{hi}CD5^{+}$	IL-35	82
	KPC 4662, KPC 2173	$CD19^{+}CD1d^{hi}CD5^{+}CD21^{hi}$	IL-35	157–159
Prostate cancer	Myc-CaP	$CD20^{+}$	Lymphotoxin	160
	Myc-CaP, TRAMP-C2	$CD19^{+}CD20^{lo}B220^{lo}IgA^{+}$PD-$L1^{+}$	IL-10	103
Squamous cell carcinoma	DMBA/TPA (inducible)	$CD19^{+}CD21^{hi}$	IL-10	70

Abbreviations: DMBA, dimethylbenz[a]anthracene; KPC, K-ras LSL.G12D$^{-/+}$, p53R172H/+, PdxCre mouse; NASH, nonalcoholic steatohepatitis; PD-L1, programmed cell death ligand 1; TPA, 12-*O*-tetradecanoyphorbol-13 acetate; TRAMP, transgenic adenocarcinoma of the mouse prostate.

suggesting that B cells negatively regulate these immune cells. Multiple mechanisms that might enhance antitumor immunity by blocking the number or function of B lymphocytes have been proposed. One challenge for understanding the function of regulatory B cells (Bregs) has been that unlike the case of Tregs, which express the canonical transcription factor FoxP3 (58), single-cell and bulk RNA-seq data have not demonstrated a lineage-defining transcription factor for Bregs. Studies have demonstrated a marked increase in the number of Bregs during inflammation, suggesting that these cells may be generated or activated in response to cytokines produced by innate immune or tumor cells such as TNF, IL-1β, and IL-6 or those such as IL-21 and GM-CSF, which are produced by adaptive immune cells, such as Th17 cells (59). Thus, Bregs are most likely not a separate lineage of B cells but rather B cells that are capable of immunosuppressive activity in a specific environment. The importance of this understanding is underscored by the work of Hu et al. (50). Using single-cell transcriptomics to evaluate B cells from breast tumor patients prior to therapy, they did not find populations of IL-10-, IL-35-, or TGF-β-expressing B cells prior to therapy.

4.1. IL-10 Bregs

B cells can generate different proteins that have immunosuppressive properties, including IL-10, IL-35, and TGF-β (**Table 2**). Additionally, both mature B cells and immature B cells as well as plasmablasts that generate immunosuppressive proteins have been characterized (60–66) (**Figure 2**). Several groups in the later part of the twentieth century demonstrated that B cells can suppress an adaptive immune response (67, 68). The Bhan group (69) published the initial description of a population of B cells that generated IL-10. They found a population of B cells that were $CD1d^{hi}CD21^{int}IgM^{int}CD23^{hi}$ and important for controlling gastrointestinal tract inflammation.

4.1.1. B10 cells. The Tedder laboratory (66) further expanded on this finding, demonstrating that B cells that express IL-10 also express CD1d and CD5. They found that a small population of such cells were present in the spleen in control mice and increased five- to sevenfold in the presence of inflammation (70). They termed them B10 cells.

It is now clear that the paradigm that characterizes B cells based on surface expression of proteins, delineating lineages of B cells such as transitional, marginal zone, and germinal center B cells, poorly delineates Bregs. Bregs have been identified in all of these lineages, as well as among the larger number of follicular B cells (71). The population of B cells described by Yanaba et al. (66) appears to overlap with these different populations of splenic B cells and may be a significant proportion of these populations. However, a significant number of Bregs do not express CD1d and CD5 and fall outside the parameters described for B10 cells. Currently, it is clear that not all B cells that express IL-10 have the surface phenotype of B10 cells.

Several groups have demonstrated a role for IL-10 production by B cells in diminishing the antitumor immune response. Tumor cells such as thymoma and melanoma cell lines can induce B cell production of IL-10, which was significantly decreased when B cells did not express CD40 or when the tumor cells did not express CD40L. The protumorigenic role of IL-10 produced by B cells was mediated by blocking expression of IFN-γ by $CD8^+$ T cells or NK cells (72).

Mechanisms to block the activation of $CD1d^+CD5^+$ B cells in the TME are currently being evaluated. One area of interest is the role of BCR signaling in the activity of these cells. Das & Bar-Sagi (73) found that the ability of CD40 and IL-6 to activate $CD1d^+CD5^+$ B cells was attenuated by blocking the function of BTK (Bruton tyrosine kinase) using tirabrutinib. Interestingly, this also blocked the generation of both IL-10 and IL-35 by these cells. They found in intraductal pancreatic cancer lesions (PanIN) increased expression of activated BTK with enhanced expression of BTK phosphorylated at tyrosine 223. Importantly, inhibiting BTK with tirabrutinib led to decreased growth of PanIN lesions in $K\text{-}ras^{G12D}$ mice. Thus, BTK inhibitors are a potential target in tumors enriched for Bregs.

4.2. Bregs Expressing IL-35

A second group of Bregs that generate IL-35 has been described. IL-35 is part of the IL-12 family of cytokines, which also includes IL-12, IL-23, and IL-27 (74, 75); IL-35 is a dimeric protein composed of the IL-12 α chain and the IL-27 β chain. These are encoded by the genes *IL12A* and *EBI3* (Epstein-Barr virus–induced gene 3), respectively. Unlike the other members of the IL-12 family, IL-35 is generated predominantly by adaptive immune cells and not innate immune cells. Tregs that express IL-35 have been characterized by different groups in both autoimmunity and cancer models (76, 77). Tumor cells have also been shown to produce IL-35, which enhanced tumor growth through multiple mechanisms, including recruitment of immunosuppressive myeloid cells (78–80). A role for B cell generation of IL-35 in modulating inflammation in autoimmune and infectious models was shown by Shen et al. (81), who demonstrated that inhibiting the generation of IL-35 by B cells leads to increased expression of IL-17A and IFN-γ by T cells. In their studies, the antigen-presenting function of B cells was diminished by B cell expression of IL-35.

Pylayeva-Gupta et al. (82) identified a critical role for the generation of IL-35 by B cells in the pathophysiology of genetically modified mouse models of pancreatic ductal adenocarcinoma (PDAC). Tumor growth was inhibited in mice lacking B cells, and this was reversed by the transfer of B cells that could make IL-10 or wild-type B cells but not B cells that expressed IL-35. These cells were also present in human PDAC tumors. These Bregs were $CD19^+CD1d^{hi}CD21^{hi}CD5^+$,

an immunophenotype similar to that described by Tedder's group for B10 cells (66, 83). They accumulated in the spleen and pancreas in mice that developed PDAC. Recent studies have characterized a similar population of IL-35-expressing B cells that express PD-L1 and that are critical to the development of cytokine-induced pancreatitis and PDAC (84).

4.3. TIM-1$^+$ Bregs

Another population of B cells with immunosuppressive properties has been defined by the expression of the protein T cell immunoglobulin domain and mucin domain (TIM-1). Ding et al. (85) demonstrated that TIM-1 was expressed on 5–8% of splenic B cells in mice and that vaccination increased this number. B cell expression of IL-4 and IL-10 was predominantly found within the TIM-1$^+$ population of B cells, and these cells were induced by antibodies specific for TIM-1. A role for TIM-1$^+$ Bregs in hepatocellular carcinoma (HCC) has been demonstrated (86). Patients with HCC had increased infiltration of TIM-1$^+$ B cells into their tumors compared to peritumoral tissue. These B cells were CD5^{hi}CD24$^-$CD27$^{+/-}$ and CD38$^{+/hi}$. TIM-1-expressing B cells generated IL-10 and suppressed the function of CD8$^+$ T cells. In correlative analyses, the presence of TIM-1-expressing B cells was associated with early recurrence, decreased survival, and higher disease stage in HCC. Tumor cell exosomes that expressed high mobility group box 1 led to the expansion of TIM-1$^+$ Bregs via signaling through Toll-like receptor 2/4 (TLR2/4) and the MAP kinase pathway. A second study demonstrated that TIM-1$^+$ B cells that generated IL-10 were critical to suppress the cytotoxic activity of CD4$^+$ T cells in hepatitis B virus–induced HCC (87).

4.4. Clinical Role of IL-10-Producing B Cells: Do Humans Generate Bregs?

Over the last decade, there has been increasing recognition of different subsets of human Bregs that control inflammation. Following up on studies using mouse models, the Tedder group characterized a population of human B cells that expressed IL-10 after stimulation with phorbol ester and ionomycin (65, 83). These cells were CD24^{hi} and CD27$^+$ and expressed CD48^{hi} and CD148^{hi}. When activated, these cells inhibited CD4$^+$ T cell expression of proinflammatory cytokines. Since that description, a number of human B cell populations that can inhibit immune responses have been characterized. De Masson et al. (88) described a fraction of IL-10-generating B cells that express CD27^{hi}/CD38^{hi}. These investigators found that patients with chronic graft-versus-host disease had decreased numbers of Bregs when Bregs were induced by TLR stimulation. The decreased generation of Bregs in these patients was associated with impaired generation of activated ERK and STAT3.

A number of recent studies show that the presence of Bregs is associated with a poorer outcome in patients with cancer (**Table 3**). Zhou et al. (89) used immunohistochemistry to stain for CD19 and IL-10 in patients with squamous cell carcinoma of the tongue. In vitro, Bregs induced the expression of FoxP3 in CD4$^+$ T cells, and the presence of Bregs was associated with inferior survival. In patients with breast cancer, investigators have demonstrated an increase in Bregs that express CD19$^+$, CD24$^+$, and CD38$^+$ and generate IL-10. This population was also shown to overexpress PD-L1, one of the ligands for the receptor PD-1 (90). There was a strong correlation between the presence of PD-L1$^+$CD19$^+$CD24$^+$CD38$^+$ Bregs, CD4$^+$FoxP3$^+$CD127$^{low/-}$ Tregs, and inferior clinical survival. Thus, there may be a feedback loop by which IL-10 production by Bregs leads to the generation of innate immune cells that enhance the persistence or number of Tregs in the TME.

Previous studies have shown that patients with more aggressive, advanced gastric cancer had an increase in the frequency of IL-35-secreting B cells in the bloodstream (79). These cells were

Table 3 Regulatory B cells identified in human cancers

Cancer type	Location	Surface markers	Effector functions	Reference
Breast cancer	Tumor	$CD19^+$	IL-10, CD25	161
	Tumor	$CD19^+$	Granzyme B	102
Cervical cancer	Tumor	$CD19^+CD5^+CD1d^+$	IL-10	162
	Tumor	$CD19^+$	Granzyme B	102
Esophageal (squamous)	Tumor	$CD19^+$	IL-10, PD-1	163
Esophageal (unspecified)	Peripheral blood	$CD19^+CD5^+$	IL-10	164
Gastric cancer	Peripheral blood and tumor	$CD19^+CD24^{hi}CD38^{hi}$	IL-10, TGF-β1	165
	Tumor	$CD19^+CD24^{hi}CD27^+$	IL-10	166
	Peripheral blood	$CD19^+$	IL-35	79
Head-and-neck squamous cell carcinoma	Tumor	$CD19^+CD24^{hi}CD38^{hi}$	IL-10, CD25	34
Hepatocellular carcinoma	Tumor	$CD19^+CD5^{hi}CD27^{hi}CD38^{dim}$	PD-1, IL-10	92
Ovarian cancer	Ascites	$CD19^+CD20^+$	IL-10	167
	Tumor	$CD19^+$	Granzyme B	102
Pancreatic ductal adenocarcinoma	Tumor	$CD20^+$	IL-35	159
	Peripheral blood	$CD19^+CD24^{hi}CD38^{hi}$	IL-35, IL-10	158
Tongue squamous cell carcinoma	Tumor, lymph node	$CD19^+$	IL-10	89

correlated with the presence of other immunosuppressive cells, such as Tregs, myeloid-derived suppressor cells, and B10 cells. Hu et al. (91) evaluated for the presence of Bregs in the tumors and circulating peripheral blood cells of patients with gastric cancer. They found increased expression of IL-10 in B cells isolated from the tumor; these B cells generated more IL-10 than B cells in blood. Advanced-stage patients had a greater number of IL-10-producing B cells than early-stage patients. B cells that generated IL-10 had increased expression of CD27 and CD38, with $CD10^-CD27^+$ B cells capable of inhibiting cytokine production by T cells.

PD-1 is upregulated by B cells after activation through the BCR. Below we discuss the function of PD-1 on memory B cells in response to immune checkpoint inhibitor therapy. Xiao et al. (92) demonstrated the presence of PD-1^{hi} B cells in patients with HCC that were $CD5^{hi}CD24^{-/+}CD27^{+/hi}CD38^{dim}$. PD-$1^{hi}$ B cells in the liver of patients with HCC did not express IL-10 after activation with TLR, anti-IgM, or CD40L. PD-1 expression was modulated by CD40L and downregulated by IL-4. Binding of PD-L1 to PD-1 on these B cells did induce IL-10. Finally, anti-PD-1 therapy in murine models of HCC enhanced tumor growth, suggesting that the type of cell in the TME that expresses PD-1 may be critical in the antitumor immune response.

4.5. IL-10 Production and the Epigenome

Investigators have yet to identify transcription factors or networks that distinguish B cells that produce IL-10. Thus, changes in the methylation status of the enhancer and promoter elements of the *il10* locus, mediated by different stimuli, may be critical in the expression of IL-10 by Bregs. Supporting this hypothesis, studies have evaluated the *il10* locus in murine splenocytes after stimulation with lipopolysaccharide, phorbol-12-myristate-13-acetate (PMA), and ionomycin (93).

When the four conserved noncoding sequences (CNS) in the *il10* locus were evaluated, there was much greater methylation of cysteines in IL-10$^-$ compared to IL-10$^+$ B cells. IL-10-producing B cells had a distinctive pattern of methylation with the mCNS-4.5 region demethylated and the mCNS-9 region partially demethylated. This partial demethylation was specific to murine IL-10-producing B cells. For human IL-10-expressing B cells, the hCNS-12.5 region was demethylated. Thus, epigenetic control of the *il10* locus in response to inflammation may be critical to the generation of Bregs.

4.6. What Induces Bregs in the TME?

In murine models, the 4T1 breast cancer line has been found to induce the expansion of a population of tumor-associated Bregs (94). This process is dependent on the generation of metabolites of the 5-lipoxygenase pathway, which includes proteins such as leukotrienes, which induce Bregs through the PPARα receptor. Other studies have shown that placental growth factor generated by glioblastoma tumor cells can induce TGF-β expression in TIL-B cells (95). Additional studies have shown that the generation of TNF by tumor cells enhances the expansion of Bregs (70).

In addition to tumor cells inducing Bregs, other cells in the TME can induce the expansion of Bregs. IL-21 is expressed by Tfh cells and critical for immunoglobulin class switching by B cells. IL-21 can enhance the number of Bregs (96). This interaction can also induce the expansion or differentiation of Bregs. Additionally, the interaction of PD-1 on B cells with PD-L1/2 expressed by tumor cells or immune cells can induce the expansion or differentiation of Bregs. Thus, it appears that multiple stimuli critical for the activation or function of B cells can enhance the generation of Bregs.

4.7. Other Methods of Immunosuppression: Murine Studies

The roles of the PD-1 receptor and the ligands that bind to it, PD-L1 and PD-L2, in the immunosuppressive TME have been well described in recent reviews (1, 2, 97). One potential mechanism for the immunosuppression of B cells is the expression in the TME of PD-L1, which can engage PD-1 expressed by adaptive immune cells. Interestingly, PD-L1 is expressed by naive B cells that are CD19$^+$CD80$^+$CD86$^+$MHC-II$^+$CD44$^+$CD69$^+$, and this phenotype may represent recently described tissue-resident B cells (98). These cells have been linked to tumor cell growth in the pleura, as they inhibit the function and reduce the number of effector T cells, such as Th17 cells (99). The generation of adenosine via the activity of the ectonucleotidases CD39 and CD73 leads to the development of an immunosuppressive TME. CD39$^+$/CD73$^+$ B cells, which can be activated by CD40L and IL-4, suppress the proliferation of CD4$^+$ and CD8$^+$ T cells, perhaps through the generation of 5′-AMP (100).

Depletion of B cells leads to diminished tumor growth in mice given the murine EMT-6 mammary tumor cell line. The mechanism for this activity involves another subset of Bregs (101). In this model, a significant percentage of B cells in the TME, but not in the spleen, expressed TGF-β1/LAP. They demonstrated that TIL-B cells can suppress proliferation of CD4$^+$ and CD8$^+$ T cells and NK cells in vitro. The expression of TGF-β1/LAP was induced by co-culture with splenic B cells in the presence of EMT-6 tumor cells. TIL-B cells expressed PD-L1, and incubation of these cells with antibodies specific for TGF-β1 or PD-L1 in part restored T cell proliferation in the presence of TIL-B cells.

Other populations of B cells are capable of suppressing antitumor immune cells. B cells that have undergone class switch recombination mediated by IL-21 expressed by Tfh cells expressed granzyme B, which was transferred to CD4$^+$ T cells, leading to degradation of the ζ chain of the T cell receptor (TCR) and loss of TCR signaling after engagement of MHC-II bound to peptide

(102). These granzyme B–expressing B cells were found in a number of human tumors, including breast, cervical, ovarian, colorectal and prostate carcinomas.

5. REGULATORY PLASMA CELLS

Various investigators have characterized populations of PCs that are immunosuppressive in different models. A population of $CD19^+CD138^+IgA^+$ cells that were characterized in the TRAMP (transgenic adenocarcinoma of the mouse prostate) model of prostate cancer (103) was critical in tumor progression after treatment with the chemotherapeutic drug oxaliplatin. Low-dose treatment with oxaliplatin, which induces immunological cell death, was not effective unless B cells were depleted. After multiple treatments with oxaliplatin, there was significant expansion of IgA-producing PCs that expressed $CD19^+$ and $CD138^+$ and were present in the TME. These cells were adjacent to CXCL13-generating stromal cells. Oxaliplatin induced the expression of IL-10, PD-L1, and TGF-β1 by these PCs. Interestingly, these cells were also found in patients with therapy-resistant prostate cancer.

6. CLINICAL PARADOX: CLEAR CELL RENAL CELL CARCINOMA

Our group evaluated the role of B cells by assessing for increased expression of B cell metagenes using samples obtained by The Cancer Genome Atlas (TCGA) (23). As discussed in this review, we found that increased expression of B cells correlated with improved clinical outcomes. However, there was a clear outlier in these data. The increased expression of B cell metagenes was correlated with a poorer outcome in patients with clear cell renal cell carcinoma (RCC). Li et al. (104) evaluated potential mechanisms for the activity of B cells in RCC. They found increased recruitment of B cells to the tumor compared to surrounding tissue. Co-culture of B cells with renal cell tumor lines induced the expression of HIF-2α, which increased the migration and invasion of tumor cells. B cells produced IL-1β, and inhibition of IL-1β led to decreased migration and invasion of RCC cell lines in vitro. Increased tumor invasion was mediated by activation of the PI3K/AKT pathway, which was mediated by IL-1β. Finally, they demonstrated that this process upregulated the Notch ligand DLL4 and activation of the Notch pathway, which was important in the migration of tumor cells. Thus, in this model, generation of proinflammatory cytokines by B cells was associated with enhanced migration and invasion by RCC cell lines.

7. ANTITUMOR ACTIVITIES OF B CELLS

7.1. B Cells and Correlation with Improved Prognosis

Like tumor-infiltrating T cells, TIL-B cells are a heterogeneous group of cells with diverse functions. In addition to their protumorigenic effects, B cells also have antitumor activity (**Figure 2**). Using immunohistochemistry to identify B cells and/or PCs or using immunogenomics to identify B cell or PC metagene signatures, researchers have found an association between improved prognosis and the increased presence of B cells in patients with melanoma, sarcoma, breast cancer, esophageal cancer, non–small cell lung cancer, colon cancer, or biliary tract cancer (105). However, while the majority of studies show an association between the increased presence of $CD20^+$ B cells and an improved clinical prognosis, this is not true for all tumors (105). For example, our group found that increased expression of B cell metagenes is associated with an inferior prognosis in patients with clear cell RCC (23).

B cell gene expression signatures have been used in multiple studies to evaluate associations with prognosis given patient information and tumor gene expression profiling data. In a foundational study in cancer immunogenomics, gene expression signatures were derived from expression

profiles of sorted leukocytes and analyzed for associations with survival in colon cancer (106). B cell and follicular helper T cell signatures were associated with improved survival. One drawback of gene signature expression–based scoring is that genes within each signature can be nonspecific for cell phenotypes of interest; genes within a gene signature representative of one cell type may be expressed by other cell types as well. To overcome this, methods have been developed to estimate frequencies of individual cell phenotypes based on gene expression data. The most widely used of these methods is CIBERSORT (cell type identification by estimating relative subsets of known RNA transcripts) (107), which revealed that PCs were associated with improved prognosis in a pooled set of cancers as well as in lung adenocarcinoma and breast cancer individually. Similarly, our pan-cancer analysis, using RNA-seq data and B cell metagene analysis, found associations between B cell gene signatures and survival in breast cancer, head-and-neck cancer, lung adenocarcinoma, and melanoma (23).

TIL-B cells are present in up to 60% of breast cancers (26), and TIL-B cell and B cell genomic signatures are associated with improved prognosis and response to chemotherapy in highly proliferative subtypes of breast cancer, such as triple-negative breast cancer (24, 108–115). Our group demonstrated, using data from TCGA, that prognosis of patients with breast cancer was improved if they had increased expression of B cell metagenes. Interestingly, we also found that prognosis was associated with somatic hypermutation and high expression of a low diversity of BCR segments, suggesting antigen recognition by the host's adaptive immune system (24). Our group and our collaborators have also found this correlation in patients with immunoreactive ovarian cancer (24), where B cell and PC infiltrates in the tumor were associated with improved prognosis (25, 33); this appears to be mediated in part by polyclonal IgA (116). We found that B cells infiltrated into the stroma of patients with high-grade serous ovarian carcinoma metastasis and that this was associated with the generation of a memory B cell response against a restricted set of antigens. PC generation of tumor-specific IgG was also shown (25). Again, these data suggest that generation of endogenous antibodies specific for tumor antigens may be critical to the antitumor immune response in specific malignancies. Interestingly, the association between antibody generation and prognosis is greatest in hormone-associated cancers in women, with our studies and those from others either strongly suggesting or demonstrating the presence of antitumor antibodies.

Multiple groups including ours have found associations between tumor-associated B cell populations in melanoma and survival (117–119). Beyond gene expression signatures, our group has shown that BCR repertoire features consistent with antigen-driven clonal expansion and somatic hypermutation in tumor-associated B cell populations have been associated with survival (23, 118, 119). B cell populations with post–germinal center BCR repertoire features have been found in other tumor types as well, including breast cancer and human papilloma virus (HPV)-positive head-and-neck cancer (50, 120). It is unknown to what extent these cells make and secrete tumor-antigen-specific antibodies that directly contribute to elimination of tumor cells, though there are encouraging data indicating that antibodies derived from patient sera, and in some cases from tumor-associated B cells, can bind tumor cells and contribute to antibody-dependent cytotoxicity.

In lung adenocarcinoma, a number of B cell gene signatures have been associated with improved survival (121, 122). In head-and-neck cancer, gene signatures for total B cells, germinal center B cells, light zone B cells, dark zone B cells, and PCs were derived from single-cell gene expression profiling and were then tested for association with progression-free survival in TCGA head-and-neck tumors (123). Overall B cell gene signature expression and germinal center B cell gene signature expression were associated with improved progression-free survival following surgical resection, whereas PC gene signatures were associated with worse progression-free survival. As the latter association was not statistically significant, it is unclear whether this negative survival association with PCs represents differential biology in head-and-neck cancer.

Recently, there has been increased interest in the study of TLSs in tumors and their association with patient prognosis. In patients with head-and-neck squamous cell carcinoma, the presence of B cell signatures and TLSs was also convincingly associated with improved prognosis, particularly in HPV-positive patients (34, 123, 124). Similarly, B cells and TLSs in non–small cell lung cancer correlate positively with prognosis (30) and are associated with increased $CD4^+$ T cell repertoire clonality (125) and a reduction in the frequency of Tregs (29), although the role of B cells may depend on both their phenotype and the phase of cancer progression (126). TLSs and their gene signatures have also been associated with improved outcomes in muscle-invasive bladder cancer (127), melanoma, sarcoma, colorectal cancer (47), lung cancer (126), and pancreatic cancer (27, 32). The breadth of tumors in which TLSs have been discovered and associated with patient prognosis is striking; however, much remains to be discovered about the biological mechanisms driving their generation within tumor tissues, what mediates their recruitment to tumor sites, and the mechanism for their correlation with antitumor immunity.

7.2. Antibodies: The Yin and Yang of Therapy

B cells can differentiate into plasmablasts and PCs that secrete antibody. These antibodies have been associated with both improved antitumor activity (see the next section) and increased tumor growth. Previous studies have shown that increased expression of circulating immune complexes, formed by the interaction of antibody with antigen, can occur in inflammatory settings (128). These complexes may result in clearance of the antigen. However, in other circumstances, they mediate increased inflammation upon their deposition in tissue, which leads to the activation of the complement pathway and engagement of innate immune cells via Fc receptors. De Visser et al. (129) demonstrated that in a model of epithelial carcinogenesis induced by HPV16 under control of the human keratin 14 promoter, the absence of mature B and T lymphocytes led to a reduction in the development of tumors that could be reversed by the transfer of either serum or B cells from $HPV16^+$ mice (129). This group demonstrated that the absence of mature B cells led to a reduction in mast cells and $Gr\text{-}1^+$ myeloid cells while increasing the presence in the tumor of both $CD8^+$ and $CD4^+$ T cells, $Gr\text{-}1^-$ myeloid cells, and $CD11c^+$ dendritic cells (130). Interestingly, they confirmed these findings in mice that lacked FcRγ but retained expression of the inhibitory FcRγII. The absence of FcRγ led to reduced angiogenesis, reduced keratinocyte proliferation, and reduced focal dysplasia, which resulted in a marked decrease in the generation of squamous cell cancer. FcRγ activation of mast cells and $CD11b^+$ macrophages by antibody was necessary for tumor development and growth. Thus, antibodies can mediate the growth of squamous cell tumors by activating FcγR on myeloid and mast cells. Similar to these findings, B cell accumulation in the TME and IgG production were associated with a poorer outcome in a model of squamous cell carcinoma (131).

7.3. Cancer Patients Can Generate Endogenous Antibodies That Bind Tumor Cell Antigens

There is direct evidence that cancer patients develop endogenous antibodies that bind cancer cells and are associated with improved clinical outcome. Antibodies against the cancer testes antigen NY-ESO-1 arise in 20–50% of ovarian cancer and melanoma patients that express this antigen (132–135). In melanoma, the presence of NY-ESO-1-specific antibody was associated with improved clinical benefit from CTLA-4 inhibition (133). Clinical benefit rate was highest in patients who had both antibody responses and antigen-specific $CD8^+$ T cell responses; those with antibody responses but without antigen-specific $CD8^+$ T cell responses had less benefit. These data suggest that antibodies were not solely biomarkers of an immune response. IgG

antibodies derived from melanoma and ovarian tumors were able to bind antigens on primary tumors and cell lines (25, 136). Antibodies derived from breast cancer TIL-B cells were found to bind β-actin that was exposed on the surface of apoptotic malignant cells (137). Using phage display libraries from TIL-B cells, Pavoni et al. (54) screened for antibodies specific for CEA, MUC1, and the ED-B domain of fibronectin. All patient samples had antibodies specific to one of these proteins. Additionally, antibodies specific to two breast cancer cell lines, MCF7 and MDA-MB-468, were found (54). Quite recently in a large cohort of patients with ovarian cancer, polyclonal IgA antibodies made by tumor-associated B cells bound IgA receptors on the surface of tumor cells, leading to transcytosis and tumor growth inhibition as well as increased killing of tumor cells by cytotoxic T cells (116). These investigators mapped the specificities of antibodies generated by ovarian cancer patients and demonstrated that these antibodies bound to hundreds of cell surface and secreted proteins (116). These studies provide proof that endogenous B cells can generate antitumor-specific antibodies, which are associated with improved clinical outcome.

7.4. Are There Tumor B Cell Neoantigens?

Recent work has indicated that T cells in patients generate immune responses to a variety of tumor antigens, with the response to nonsynonymous single-nucleotide variants being the most commonly studied (138, 139). Thus, driver or passenger mutations can induce an antitumor immune response. However, there are other approaches to generate neoantigens. These include presentation of novel protein-coding regions that result from genetic translocations or splicing variants (140). Our group has shown that in clear cell RCC, immune responses to endogenous human retroelements that are overexpressed in these tumors correlated with outcome of checkpoint inhibitor therapy, with several patients having significant numbers of T cells specific for human retroelements (141, 142). Given that our group and others have found antibodies specific for tumor antigens in patients, are any of these specific for tumor-specific neoantigens? Given the large number of neoantigens that can be generated from spliced or single-nucleotide variants in tumors with high mutation burdens, it would be quite unusual if none of those antigens could be recognized by B cells. Our group has begun to evaluate for the presence of tumor-specific neoantigens using a combination of computational informatics, single-cell sequencing, and antibody cloning, with a focus on patients with breast or ovarian cancer. It will be quite interesting to determine whether these antitumor immune responses are private, as found overwhelmingly in neoantigen-specific T cell responses, or whether these are immune responses to public neoantigens that are present in different patients with cancer.

8. B CELLS AND RESPONSE TO CHECKPOINT BLOCKADE

The expression of checkpoint molecules on T cells has been well described. B cells express checkpoint molecules including PD-1, PD-L1/2, and CTLA-4. In the last year, multiple groups have defined a critical role for B cells in the response to ICI in cancer. In preclinical models of triple-negative breast cancer, we and our colleagues in the Perou laboratory (143) showed that B cell–mediated activation of T cells and antibody production played a critical role in the response to ICI in murine models of high-mutational-burden triple-negative breast cancer; dual checkpoint blockade had significantly diminished efficacy after B cell depletion and in a murine model where B cells were unable to secrete antibody (143). B cell antibody production was dependent on Tfh cells, and T cell memory correlated with the presence of B cells. Additionally, in mice treated with ICI, both the TCR and BCR repertoires were clonally restricted. In studies of human melanoma, B cells predict response to ICI therapy, in part through enhancing T cell function through recruitment and activation of PD-1^+ T cells (117). Patients with melanoma who respond to ICI

therapy have clonal expansion and increased numbers of memory B cells, with B cells localized within TLSs (31). Interestingly, however, circulating inflammatory B cells that generate TNF or IL-6 are associated with failure to respond to anti-CTLA-4 checkpoint blockade in melanoma, again highlighting the heterogeneity of B cells and their differential impact on antitumor immunity (28, 144). In patients with sarcoma, B cells were the strongest prognostic marker for response to ICI therapy (145), specifically in patients classified as having immune-high subtype sarcomas. Most recently, increasing TIL-B cells was found to be associated with improved response to ICI therapy in patients with non–small cell lung cancer (146–148).

The generation of antibody may also be important in a robust immune response in the setting of ICI therapy. Eradication of large melanomas in mice required the combination of an antigen-specific antibody with enhanced engagement of tumor integrins, given with anti-PD-1 monoclonal antibody, a long-acting IL-2, and a peptide-epitope-specific T cell vaccine (149).

9. B CELLS AND IMMUNE-RELATED ADVERSE EVENTS

Perhaps not surprisingly, B cells have also been shown to be predictors of immune-related adverse events (IRAEs) during ICI therapy. Patients who experience IRAEs during ICI therapy have early changes in their circulating B cells, with a decrease in the number of circulating $CD21^{lo}$ B cells but an increase in clonally restricted B cells and PCs (150). In a study of combinatorial ipilimumab and intralesional BCG (bacillus Calmette-Guérin) for advanced melanoma, IRAEs were associated with a significant increase in autoantibodies to self-antigens and cancer antigens, detected prior to the development of IRAE symptoms (151). Given that distinct pretreatment antibody profiles are associated with IRAEs, it has been hypothesized that pretreatment antibodies may predict subclinical autoimmunity and the development of clinical IRAEs during ICI therapy (152). However, whether IRAEs are due to existing autoimmunity or are new events remains unclear (153). In several studies, IRAEs are also associated with response to ICI therapy, although not uniformly, and the mechanism that accounts for this finding is poorly understood (3, 154).

10. CONCLUSION

As we have described, B cells have a multitude of functions in the TME. In most of the larger-population studies, the presence of B cells, assessed using either genomics or cellular approaches, has been associated with an improved outcome in cancer patients. In these instances, it is not clear whether these B cells are biomarkers for a productive immune response or critical effector cells of that response. However, the data regarding the association of clinical outcome with the generation of antibodies and our murine data showing that dual immune checkpoint inhibitor therapy is not effective for breast cancer in mice that do not secrete antibody strongly suggest that antibody generation is important in the antitumor immune response. However, it is critical that future work confirm that clinical outcome is correlated with antibody-secreting cells in the TME and characterize the antigens recognized by these antibodies. B cells, both in animal models and in humans, have also been associated with poor clinical outcomes and impaired antitumor immunity, predominantly through the generation of immunosuppressive cytokines. Most recently, a large number of groups have shown an association between TLSs and improved clinical outcome, although both the mechanism for the development of TLSs and how they function in the antitumor immune response are not clear. Future work should provide a better understanding of the role of TLSs in antitumor immunity, characterize the specificity and function of antitumor antibodies, and clarify the mechanisms that lead to Breg generation in patients and the role of Bregs in suppressing immune responses.

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