








PERSPECTIVE

Cancer Focus

B cells and cancer: To B or not to B?

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Whereas T cells have been considered the major immune cells of the tumor microenvironment able to induce tumor regression and control cancer clinical outcome, a burst of recent publications pointed to the fact that B cells may also play a prominent role. Activated in germinal centers of tertiary lymphoid structures, B cells can directly present tumor-associated antigens to T cells or produce antibodies that increase antigen presentation to T cells or kill tumor cells, resulting in a beneficial clinical impact. Immune complexes can also increase inflammation, angiogenesis, and immunosuppression via macrophage and complement activation, resulting in deleterious impact.

Introduction

At the beginning of the millennium, it was demonstrated that the immune tumor microenvironment (TME), particularly the density, localization, and functional orientation of T cells, is of paramount importance for controlling tumor growth and spread (Galon et al., 2006; Fridman et al., 2012, 2017). It established the rationale for paradigm-changing immunotherapies using antibodies against immune checkpoint inhibitors (ICIs), such as CTLA-4, PD-1, and PD-L1, that have revolutionized the treatment of cancers (Sharma and Allison, 2015). Since these therapies aim to reinvigorate exhausted T cells, it is not surprising that they are mostly effective in highly mutated tumors, which are more likely to express more tumor-specific neoantigens, resulting in a strong T cell infiltrate, such as non-small cell lung cancer (NSCLC), melanoma, and microsatellite instable tumors (Rizvi et al., 2015). However, despite being very efficient and inducing long-term responses in many cancer types, most patients are resistant to ICI therapies (Hirsch et al., 2019), urging for the identification of other components of the immune TME that are involved in tumor control and may provide novel therapeutic approaches. In this respect, the role of B cells has, until recently, been underestimated. In fact, it had been reported in some murine models that B cells, and the antibodies they produce, may favor cancer occurrence and spread. In recent years, however, studies in human cancers showed that the density of B cells, particularly in tertiary lymphoid structures (TLSs) in the tumor-adjacent TME, correlates with favorable prognosis and

predicts therapeutic response to ICIs even in tumors with low tumor mutational burden. We will put in perspective the different roles of B cells in cancer immunity, with emphasis on TLSs, and discuss the mechanisms underlying their effects and their use as prognostic biomarkers and therapeutic targets.

B cells in tumor immunology: Lessons from murine models

The question of the impact of B lymphocytes in the immune control of tumors has been addressed in several murine models, unraveling potential mechanisms of action of B cells in cancer immunity. In a transgenic mouse model of multistage epithelial carcinogenesis in genetically invalidated RAG1 mice, the group of L.M. Coussens (de Visser et al., 2005) observed a high reduction of innate cells infiltrate in premalignant skin and carcinoma incidence. Both were restored upon transfer of B cells or serum from tumor-bearing immunocompetent mice. The mechanism underlying these effects is that B lymphocytes are activated in tumor-developing mice and produce antibodies that deposit in the precancerous lesions, fueling chronic inflammation through Fcγ receptor (FcγR) activation of innate cells migrating into the preneoplastic and neoplastic TME. Whereas immune complexes did not activate complement in this model (Medler et al., 2018), complement was found to contribute to protumoral effects through antibody-driven chronic inflammation in CMT and TC1 lung cancer models (Roumenina et al., 2019a; Kwak et al., 2018). Immune complexes may also increase angiogenesis via the induction of vascular endothelial

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growth factor production by activated macrophages (Tan and Coussens, 2007). In addition, B cells may inhibit T cell responses in particular via the production of immunosuppressive cytokines (DeNardo et al., 2010). In models of fibrosarcoma and breast cancer (BC), B cell depletion with anti-IgM antibodies highly reduced the incidence of metastases as compared with control mice (Brodt and Gordon, 1978; Barbera-Guillem et al., 2000). In genetically invalidated deficient B cell mice, the growth of several types of tumors, including B16 melanoma, EL4 thymoma, and MC38 colon carcinoma, was also reduced (Qin et al., 1998; Shah et al., 2005). This effect was attributed to the lack of inhibition of the antitumor T cell response by B cell-produced cytokines such as IL-10 (Inoue et al., 2006). Finally, in a model of inflammation-driven hepatocellular carcinoma (HCC), B cell-rich TLSs were found to serve as a niche protecting tumor progenitor cells and favoring the growth of malignant cells via the production of lymphotoxin β (Finkin et al., 2015).

Altogether, these observations led to a “bad reputation” for B cells in cancer and, following several decades of unsuccessful searching for protective spontaneously produced antitumor antibodies, reinforced the paradigm that T cells, particularly CD8⁺/cytotoxic T cells, were the major, if not the only, component of beneficial antitumor immunity. A few reports challenged this statement, such as the observation that treatment of tumor-bearing mice with a B cell-depleting anti-CD20 antibody resulted in a large (more than twofold) increase in B16 melanoma volume and number of lung metastases. In this model, B cell depletion impaired induction of IFN- γ -producing T helper type 1 (Th1) cells (DiLillo et al., 2010), supporting the concept that B cells may be crucial for generating efficient T cell immunity. In conclusion, as in humans, where each patient is unique, in mice, each model is different and speaks for itself.

B cells in human cancers

The impact of B cells in cancer has been largely unraveled in studies performed in human tumors. The observation that CXCL13, a chemoattractant for B lymphocytes, is a major determinant of favorable prognosis suggests that the presence of B lymphocytes may be crucial. Thus, in colorectal cancer (CRC), an unsupervised screening for the impact of chemokine gene expression on cancer outcome revealed that tumors lacking CXCL13 had fewer intratumoral B cells and a worse prognosis than tumors expressing it (Bindea et al., 2013). The CXCL13 signature was also associated with favorable prognosis in melanoma (Helmink et al., 2020). In soft tissue sarcoma (STS), a high B cell signature significantly correlated with longer overall survival (OS), independently of the histology of the tumors, whereas there was no correlation between T cell signatures and OS. The B cell signature was the best predictor of OS, even when combined with CD8, PD-1, or CTLA-4 signatures (Petitprez et al., 2020). The fact that T follicular helper cells, which play a major role in B cell activation, correlate with favorable prognosis in breast (Gu-Trantien et al., 2017) and head and neck (Cillo et al., 2020) carcinoma also militates for a role of B cells in tumor immunity.

Direct evidence for the impact of B cells came from studies showing that their intratumoral density is associated with good

prognosis in BC (Mahmoud et al., 2012), CRC (Edin et al., 2019; Berntsson et al., 2018), NSCLC (Germain et al., 2014), head and neck cancer (van Herpen et al., 2008), ovarian cancer (Nielsen et al., 2012; Milne et al., 2009; Santoiemma et al., 2016), biliary tract cancer (Goepfert et al., 2013), primary cutaneous melanoma (Garg et al., 2016), metastatic melanoma (Cabrita et al., 2020), and HCC (Shi et al., 2013; Garnelo et al., 2017). In CRC, B cells were found associated with good outcome (Edin et al., 2019; Berntsson et al., 2018). Analysis of clonal diversity of B cell infiltrates showed that decreased diversity was associated with improved survival in primary cutaneous melanoma but with diminished OS in renal cell carcinoma (Iglesia et al., 2014, 2016; Selitsky et al., 2019). Such clonal expansion is suggestive, but not demonstrative, of the presence of anticancer B cells and does not inform about their specificity. The presence of plasma cells in BC (Yeong et al., 2018), at the vicinity of CD8T cells in ovarian cancer, and the expression of their signature in breast (Seow et al., 2020) and ovarian cancer (Nielsen et al., 2012) were associated with favorable outcome (Table 1 and Fig. 1). However, an analysis of 54 cohorts of encompassing 25 cancer types revealed that although the prognostic impact of tumor-infiltrating B cells was positive in 50% of the studies, it was deleterious or neutral in 9% and 41%, respectively (Wouters and Nelson, 2018). A few studies addressed the question of the role of B regulatory cells (B reg cells) in human cancers. The frequencies of CD19⁺-IL-10⁺ B reg cells correlated with shorter OS in bladder cancer (Zirakzadeh et al., 2020) and BC (Murakami et al., 2019), and the coexistence of B reg cells with regulatory T cells (T reg cells) correlates with shorter metastasis-free survival in BC (Ishigami et al., 2019).

Intratumoral B cells undergo isotypic switch and produce IgG or IgA antibodies directed against tumor antigens upon in vitro culture, as shown in NSCLC (Germain et al., 2014) and ovarian cancer (Montfort et al., 2017). The impact of the isotypes of antibodies produced by intratumoral B cells is critical for their Fc-dependent activities, which can be mediated through Fc receptors or complement activation. Indeed, by activating natural killer (NK) cells or macrophages via antibody-dependent cellular cytotoxicity (ADCC; Clynes and Ravetch, 1995) and macrophages via antibody-dependent cellular phagocytosis (ADCP; Gül and van Egmond, 2015), IgG antibodies may participate in antitumor activity, locally and systematically, as illustrated by the favorable prognostic impact of circulating IgG anti-MUC1 antibodies in breast, pancreatic, and gastric cancers (Hamanaka et al., 2003; Kurtenkov et al., 2007; Fremd et al., 2015). Analysis of RNA-sequencing data from cutaneous melanoma tumors from The Cancer Genome Atlas also showed an association between high levels of IgG1/IGH transcripts and favorable prognosis (Bolotin et al., 2017). In contrast, in clear cell renal cell cancer, tumor-bound IgG participates in the activation of the classical complement pathway in situ, fueling chronic inflammation and yielding poor prognosis. This occurs through the binding of complement component C1q, originating from macrophages, on tumor-associated IgG antibodies. Recruitment of tumor cell-produced C1r, C1s, C4, C2, C3, and C5 allows complement activation and chronic inflammation (Roumenina et al., 2019a). A protumoral role of B cells has been also found in squamous cell carcinoma (Affara et al., 2014) occurring by

Table 1. **Prognostic impact of B cells and TLS in human cancers**

Cancer type	Number of cases	Result	Reference
Biliary tract cancer	323	CD20 and CD8 infiltration correlate with improved OS	Goepfert et al., 2013
BC	60	The B cell-attracting chemokine CXCL13 correlates with PFS long-term survival	Gu-Trantien et al., 2017
BC	1,200	CD20 ⁺ infiltration correlates with DSS and PFS	Mahmoud et al., 2012
BC	269	CD38 ⁺ plasma cells infiltration correlates with better disease-free survival, but not OS	Yeong et al., 2018
BC	489	Coexistence of B reg and T reg cells in TLS correlates with shorter metastasis-free survival	Ishigami et al., 2019
BC	728	B cell signatures correlates with improved PFS	Iglesia et al., 2016
ccRCC	526 (TCGA); 28	B cell lineage signature demonstrates no impact on survival; B cell signature correlates with response to ICIs	Helmink et al., 2020
CRC	107	B (CD20) cell and T (CD8) cell infiltration and B cell-attracting CXCL13 chemokine correlate with longer PFS	Bindea et al., 2013
CRC	351 stage II and III	TLS density correlates with longer PFS in stage II, but not stage III, patients	Di Caro et al., 2014
CRC	557	CD20, CD138, and IGKC infiltration correlate with improved OS	Berntsson et al., 2018
CRC	316	High infiltration of CD20 ⁺ B cells correlates with improved DSS and slightly increases the prognostic effect of CD8 ⁺ T lymphocytes while having no prognostic effect on patients with tumors poorly infiltrated by CD8 ⁺ cytotoxic T lymphocytes	Edin et al., 2019
Gastric cancer	59	IL-10 ⁺ CD19 ⁺ cells are associated with shorter survival	Murakami et al., 2019
Gastric cancer	226	Majority of B cells in gastric cancer are located in TLSs and associated with better OS	Yamakoshi et al., 2020
Gastrointestinal stromal tumors	111	TLS density (H&E) correlates with longer OS and PFS	Lin et al., 2020
HCC	462	TLS density (H&E) correlates with longer OS and PFS	Li et al., 2020a
HCC	273 and 226	Density of mature TLS (H&E) and TLS signature associated with lower risk of early recurrence	Calderaro et al., 2019
HCC	120 and 200	CD20 ⁺ B cells located in the invasive margin colocalize and correlate with CD8, and a positive correlation is found between the density of B cells and OS or RFS; simultaneously, high densities of CD20 ⁺ and CD8 ⁺ cells are significantly associated with both prolonged OS and recurrence-free survival	Shi et al., 2013
HCC	112	Density of tumor-infiltrating CD19 ⁺ B cells correlates with superior survival; CD20 and CD3 correlates with high intratumoral densities of both CD20 ⁺ B cells, and CD3 ⁺ T cells had longer survival compared with those with low densities of both subsets	Garnelo et al., 2017
Head and neck cancer	30	Peritumoral B cell infiltration correlates with prolonged OS irrespective of IL-12 treatment	van Herpen et al., 2008
Lung	118	Follicular CD20 ⁺ B cells and TLS density correlate with longer DSS	Germain et al., 2014
Lung	138	TLSs are associated with good prognosis in untreated lung squamous cell carcinoma; corticosteroids may be detrimental to TLS formation	Siliņa et al., 2018
Melanoma, primary cutaneous	82	Mature DC/T cell aggregate density correlates with favorable prognosis	Ladányi et al., 2007
Melanoma	25	Frequency of plasmablast-like cells and naive B cells in pretherapy melanomas is higher in patients responding to ICB	Griss et al., 2019
Melanoma	164	CD20 and CD8 infiltration correlates with improved survival; a CD20 ⁺ and CD8 ⁺ cell combination is the best prognosticator of survival; TLS gene signature predicts prognosis and response to ICI	Cabrita et al., 2020
Melanoma	136, 28, and 21	B cell lineage signature demonstrates prolonged OS; CD20 ⁺ , B cell signature, and TLS densities predict response to ICI	Helmink et al., 2020

Table 1. **Prognostic impact of B cells and TLS in human cancers (Continued)**

Cancer type	Number of cases	Result	Reference
Melanoma, primary cutaneous	98	CD20 infiltration associated with longer OS	Garg et al., 2016
Melanoma, primary cutaneous	473	Memory B cell score and increased clonality of the BCR repertoire are associated with longer OS; lack of an assembled BCR in pretreatment tumor tissues is associated with a lack of antitumor response to a CTLA-4 inhibitor; B reg cell score is associated with shorter OS and lack of response to CTLA4	Selitsky et al., 2019
Lung	118	Follicular CD20 ⁺ B cell and TLS density correlates with longer DSS	Germain et al., 2014
Oral cancer	65	TLS density correlates with longer PFS and OS	Li et al., 2020b
Ovarian cancer	172	Tumor-infiltrating plasma cells are associated with TLSs, cytolytic response, and favorable prognosis	Kroeger et al., 2016
Ovarian cancer	194	CD20 ⁺ infiltration improves the favorable impact of CD8 ⁺ T cells on DSS; tumors positive for both CD8 and CD20 TIL showed markedly greater DSS compared with those positive for CD8 TIL alone	Nielsen et al., 2012
Ovarian cancer	199	CD20 ⁺ and CD8 ⁺ immune infiltrates and the combination correlate with improved DSS	Milne et al., 2009
Ovarian cancer	135	CD20 infiltrate correlates with improved 5-yr and 10-yr survival	Santoiemma et al., 2016
Ovarian cancer, omental metastases	92	B cells support the development of antitumor immune response	Montfort et al., 2017
Pancreas	534	TLS density (H&E) correlate with longer DSS	Hiraoka et al., 2015
Several tumor types	3,485	B and T cell signatures correlate with improved OS in BC (821), lung cancer (267), and melanoma (329); BCR diversity associated with OS in melanoma (329) and RCC (461)	Iglesia et al., 2016
STS	496 and 47	B cell lineage signature associated with longer OS independently of T cell signature and PD1, PD-L1, and Foxp3 gene expression; TLS-rich group predicts PFS and response to ICBs independently of STS histology	Petitprez et al., 2020
Urothelial urinary bladder cancer	31	CD20 ⁺ B cells in follicle-like structures correlate with longer survival; B reg cells may negatively affect prognosis	Zirakzadeh et al., 2020

ccRCC, clear cell renal cell cancer; DSS, disease-specific survival; ICB, immune check-point blocker; IGKC, immunoglobulin K constant domain; RFS, relapse-free survival; TIL, tumor-infiltrating lymphocytes.

deposition of IgG-containing immune complexes that foster FcγR-dependent activation of myeloid cells and fuel inflammation. In NSCLC, the classical pathway is activated in part in an IgM-dependent manner (Kwak et al., 2018), conferring poor prognosis (Ajona et al., 2013). The impact of antitumor IgA antibodies on patient prognosis seems deleterious, as suggested by analysis of IgA/IgGH transcripts in melanoma and in situ analyses in melanoma and bladder cancer (Bosisio et al., 2016; Welinder et al., 2016). In HCC, IgA⁺ B cells inhibit cytotoxic T cell responses that prevent hepatocarcinogenesis in the inflamed liver (Shalapour et al., 2017). The impact of B cells, although undoubtful, may therefore be a tumor contexture-dependent double-edged sword.

B cells and TLSs

Comprehensive analyses of the TME in different human cancers led to the observation that in human tumors, B cells were mostly located in TLSs. TLSs are ectopic lymphoid organs that develop in inflamed tissues in the context of chronic antigen stimulation. In cancers, proimmunogenic inflammation can also be induced or increased by treatments such as chemotherapy (Lu et al.,

2020; Kuwabara et al., 2019) or radiotherapy (Boivin et al., 2018). TLSs are formed in inflamed sites, starting upon contact of IL-7-secreting stromal cells with tissue-resident monocytic cells, Th17 cells, or B cells in a CXCL13-rich milieu (Buckley et al., 2015; Nayar et al., 2016; Barone et al., 2016; Jones et al., 2016). CCL21 and CXCL12 participate in lymphocytes recruitment, while CXCL13 and CCL19, together with adhesion molecules, govern the structural organization of the forming TLSs (Pitzalis et al., 2014). TLSs are sites of generation of immune responses to locally produced antigens, developing even in the absence of lymph nodes in lymphotoxin α invalidated mice (Moyron-Quiroz et al., 2006). Mature TLSs contain a T cell zone, where mature dendritic cells (DCs) present antigen to T cells, and a prominent B cell zone organized in a germinal center (GC), with follicular DCs (FDCs) and proliferating B cells, expression of activation-induced deaminase, and BCL6 allowing class switch and maturation toward plasma cells (Sautès-Fridman et al., 2019). They are surrounded by high endothelial venules (Ager, 2017).

In 2008, it was reported that TLSs were present in NSCLC tumors and that their density correlated with favorable

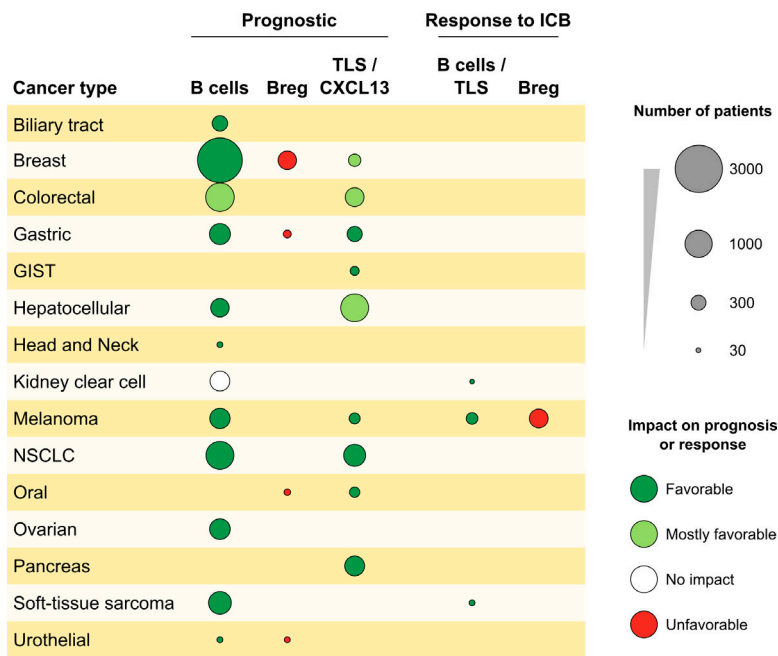


Figure 1. **Impact of B cells and TLSs on prognosis and response to ICIs.** Analysis of 15 different tumor types. The surface of the spot is proportional to the number of patients for each cancer, with a favorable impact (green), mostly favorable impact (light green), unfavorable impact (red), or no impact (white) on PFS or OS and therapeutic response to ICIs.

prognosis (Dieu-Nosjean et al., 2008). Subsequently, it was shown that T cells in TLS⁺ tumors presented a Th1/cytotoxic functional orientation (Goc et al., 2014) and that B cells were also activated in TLS GCs to proliferate and differentiate into plasma cells producing antibodies to tumor-associated antigens. A high density of B cell follicles correlated with longer progression-free survival (PFS) and OS in NSCLC (Germain et al., 2014). The presence of TLSs was reported in many cancer types, including melanoma (Ladányi et al., 2007), bladder (Zirakzadeh et al., 2020), colorectal (Di Caro et al., 2014; Posch et al., 2017), gastric (Yamakoshi et al., 2020), gastrointestinal stromal tumor (Lin et al., 2020), HCC (Li et al., 2020a; Calderaro et al., 2019) ovarian (Kroeger et al., 2016), oral (Li et al., 2020b), squamous lung (Siliņa et al., 2018), and pancreatic carcinomas (Hiraoka et al., 2015; Table 1 and Fig. 1; reviewed in Sautès-Fridman et al., 2019). In general, the presence of TLSs inside or adjacent to tumor nests correlated with favorable prognosis (Sautès-Fridman et al., 2019). The degree of TLS maturation seems to be important, since GC-containing TLSs were the best predictors of lack of cancer recurrence in stage II/III CRC (Posch et al., 2017), squamous cell carcinoma (Siliņa et al., 2018), and HCC (Calderaro et al., 2019), whereas precancerous lesions with immature TLSs progressed toward malignancy (Meylan et al., 2020). Indeed, in premalignant early stages of HCC, TLSs found in high-grade dysplastic and early HCC nodules were immature, in the form of lymphoid aggregates without a fully matured GC. Their presence correlated with markers of inflammation, immunosuppression, and immune exhaustion, which may favor transition to HCC (Meylan et al., 2020). This finding is reminiscent of the work of Finkin et al. (2015) showing that TLSs may serve as niches protecting tumor cells in a murine model of HCC. Together with the reports in murine models of premalignant stages (de Visser et al., 2005), they support a different impact of TLSs and B cells according to the stage of cancer. Altogether, these studies indicate that TLSs may shape cancer-controlling

immunity and that B cells inside GCs are important in this process and influence clinical outcome.

B cells predict therapeutic response to immunotherapy by ICIs

Whereas immunotherapy using antibodies to ICIs aims to reinvigorate effector T lymphocytes, several publications pointed to the fact that B cells may be major players of therapeutic efficacy.

In STS, B cell and plasma cell signatures were pathognomonic of an immune-rich sarcoma immune class also characterized by the presence of TLSs. Patients with this sarcoma immune class highly responded (50%) to pembrolizumab, an anti-PD-1 antibody, whereas none of the patients from immune desert classes responded (Petitprez et al., 2020). This finding may change the medical care of STS patients, who are considered poor responders to ICIs. It has generated a prospective clinical trial in which patients to be treated are selected on the basis of B cell rich TLS (ClinicalTrials.gov identifier NCT02406781). In melanoma, TLS and B cell signatures, and not T cell signatures, predicted therapeutic responses to pembrolizumab and ipilimumab, an anti-CTLA-4 antibody. B cells in tumors of responding patients exhibited oligoclonal repertoires of the Ig genes as compared with the polyclonal B cell repertoires of nonresponding patients. Moreover, B cells and TLS densities increased during treatment in responding, but not nonresponding, patients (Helmink et al., 2020), and a TLS gene signature synergized with a T effector signature to predict responses to ICIs with anti-PD-1 and anti-CTLA-4 antibodies (Cabrita et al., 2020). In contrast, a B reg cell signature was associated with lack of response to anti-CTLA-4 in cutaneous melanoma (Selitsky et al., 2019).

Not all B cell subtypes are likely to participate in response to ICIs, and recent data suggest that plasmablasts are in particular more frequent in responder patients (Griss et al., 2019). Indeed, multiomics predictions are not absolute, but they provide

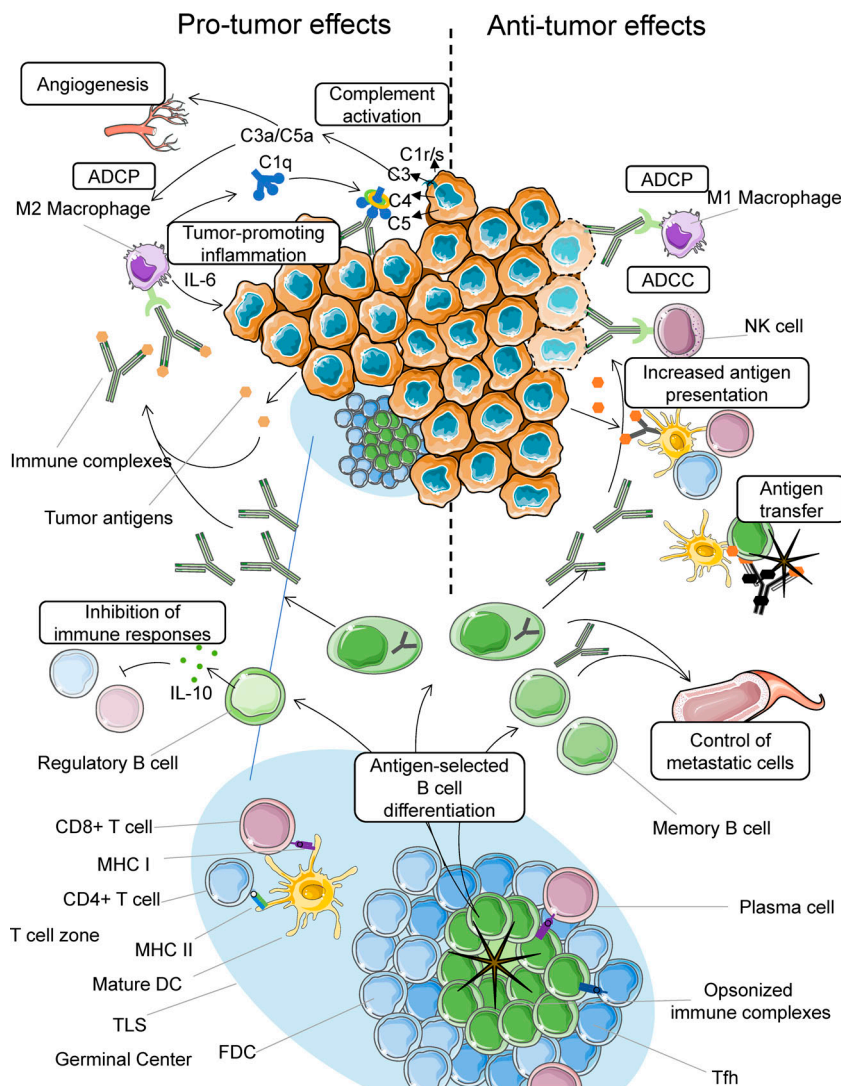


Figure 2. Mechanisms of action of B cells in cancer. In GCs of TLSs, B cells are selected by antigen presented by FDC-associated immune complexes. They are activated with the help of T follicular helper (Tfh) cells, proliferate, and differentiate into memory B cells and plasma cells. B cells present antigens to CD4 and CD8 T cells. Plasma cells produce IgG antibodies, which may increase antigen presentation to T cells after uptake of immune complexes by DCs and kill or engulf tumor cells via ADCC and ADCP, respectively (right). B cells can also transfer antigen directly to DCs or via complement receptor 2 to FDCs in the form of opsonized immune complexes. Memory B cells and antibodies circulate in the blood, where they help control potential metastatic cells (right). In contrast, binding of immune complexes to macrophages results in their activation and the production of proinflammatory mediators that exert pro-tumor activities. Tumor cell-bound antibodies can also activate complement, which fuels inflammation and activates endothelial cells, promoting tumor growth and spread (left). B reg cells may also inhibit immune responses via the production of IL-10.

significant predictive correlations that open a new field of investigations integrating B cells as major players and potential targets of novel immunotherapeutic approaches. Single-cell transcriptomics analysis of tumor-infiltrating B cells allowed for the identification of transcriptomic programs that are specifically expressed in B cell-infiltrating ICI-responsive tumors (Helmink et al., 2020). Such analyses may pave the way to better understand how each B cell subtype contributes to patient survival and response to ICIs.

B cells and cancer: Mechanisms of action

The functions of B cells are multiple. Although a truly mechanistic aspect of their impact is still missing, Fig. 2 illustrates potential mechanisms that may operate in the participation of B cell to tumor immunity.

Antigen presentation

B cells recognize and internalize native proteins and glycoproteins via the B cell receptor (BCR). They internalize the proteins thanks to the immunotyrosine activation motif of the CD79 α and β signaling chains associated with the antigen-

recognizing Ig within the BCR. Subsequent cytoplasmic processing of the proteins allows antigenic peptide association with MHC class II molecules and presentation of the complex to CD4 T helper cells and with MHC class I-associated peptides to CD8T cells. This mechanism of T cell activation occurs not only in lymph nodes but also in TLSs (Bruno et al., 2017; Garaud et al., 2019; Wouters and Nelson, 2018). Close contact between BCR-bound antigen and DCs favors antigen transfer to the latter and supports efficient antigen presentation to T cells (Harvey et al., 2014). In addition, B cells can capture immune complexes via complement receptors and transfer these antigens to FDCs (Phan et al., 2007), increasing the GC response. In tumors, B cells may therefore enhance tumor-associated antigen presentation to proximal T cells, resulting in a stronger T cell response.

In TLS GCs, plasma cells are generated and produce IgG antibodies to tumor-associated antigens, and the immune complexes formed are internalized by DCs, which process the antigens and present them in a very efficient way to CD4 and CD8 T cells. It is known that the quantity of antigen necessary to induce a T cell response is much lower (1,000-10,000 times) when internalized via an immune complex than its native

counterpart (Kalergis and Ravetch, 2002). This mechanism may explain why B cells are crucial to obtain efficient T cell responses in poorly mutated tumors in which the antigenic load is low and therefore not sufficient to directly activate T cells. It may be the case in STS, ovarian cancer, or renal cell carcinoma. It may also amplify T cell responses in tumors with high tumor mutational burden such as melanoma or NSCLC.

Another important modulator of B cell activation is complement, which can be activated on dying tumor cells, as recently shown in BC tumors in response to chemotherapy. Through binding to CR2 expressed by B cells, complement cleavage product C3b induces the generation of an ICOS-L⁺ B cell subset that boosts T cell immunity by enhancing tumor-specific CD8T cells and the Th1/T reg cell ratio. Emergence of this B cell subset is associated with improved therapeutic efficacy of neoadjuvant chemotherapy in BC, particularly in triple-negative tumors, and prolonged survival of patients (Lu et al., 2020; Sautès-Fridman and Roumenina, 2020).

Antibody production

In addition to amplifying T cell immunity via antigen presentation, antibodies produced by plasma cells in TLS GCs exert effector functions. Antibodies directed against tumor-associated antigens such as LAGE-1, MAGE antigens, and NY-ESO-1 were detected in supernatants of tumor-infiltrating B cells from half of the NSCLC patients (Germain et al., 2014). Antibodies against the tumor-associated antigen MUC1 overexpressed in tumors under an unglycosylated form and against ganglioside GD3, CEA, MUC1, and FN1 have been detected in BC (Montfort et al., 2017; Coronella et al., 2002; Garaud et al., 2019; Pavoni et al., 2007). These are tumor-associated rather than cancer-specific antibodies (such as mutated RAS), but they may be efficient in antitumor responses, since they recognize and bind to membrane antigens expressed by tumor cells. One of the interests of B cells for responses to immunotherapy is that although effector T cells almost always recognize patient's selective private neoantigens (Tran et al., 2017), B cells and the antibodies they produce may recognize shared tumor-associated antigens (Heesters et al., 2016). IgG antibodies bind to FcγRs on NK cells and macrophages, activating them to destroy tumor cells via ADCC.

ADCC killing of target cells is very efficient, even when these cells have a low antigen load. It may, however, not be very efficient in solid tumors, as NK cells are scarce and often anergic (Platonova et al., 2011). Macrophages, which are often the most prominent tumor-infiltrating hematopoietic cells, may act as effector cells, inducing the killing of tumor cells via ADCC (Clynes and Ravetch, 1995) or their phagocytosis via ADCP (Gül and van Egmond, 2015). Following ADCP, macrophages may also up-regulate PD-L1 and indoleamine 2,3-dioxygenase and support local immunosuppression (Su et al., 2018). In addition, macrophages may be immunosuppressive cells through the production of proangiogenic (vascular endothelial growth factor) or immunosuppressive (TGF-β) cytokines (Campa et al., 2015; Amornsiripanitch et al., 2010). By activating macrophages, IgG immune complexes may also support chronic inflammation (Clynes and Ravetch, 1995; Sylvestre et al., 1996), angiogenesis, and immunosuppression, thus favoring tumor growth. A

similar mechanism may operate when tumor cell-bound IgG activated locally produced complement with the production of the anaphylatoxins proangiogenic and proinflammatory complement components C3a and C5a (Roumenina et al., 2019b). Complement activation could also lead to tumor cell killing, but it seems unlikely, since malignant tumor cells in solid tumors are generally equipped with complement-inhibiting molecules (CD46, CD55, and CD59; Roumenina et al., 2019b). Interestingly, antibodies against the complement regulator factor H (FH) were found in patients with NSCLC and were shown to protect against tumor progression (Campa et al., 2015; Amornsiripanitch et al., 2010). These antibodies recognize a FH neoantigen produced by the tumor cells. B cells from anti-FH-positive patients were sorted and used to select an antibody with therapeutic properties in mouse models (Bushey et al., 2016). Although it was suggested that the anti-FH antibodies stimulate complement-mediated cancer cell killing, the classical pathway is deleterious in NSCLC (Ajona et al., 2013; Kwak et al., 2018), indicating that further studies are needed to unravel the complex mechanisms by which the B cells and produced Ig affect tumor progression.

In general, the IgA isotype is often characteristic of the B reg cell/T reg cell circuit that regulates mucosal inflammation and T reg cells producing TGF-β, which mediates isotype class switching to IgA (Stavnezer and Kang, 2009). In HCC, IgA⁺ B cells inhibit cytotoxic T cell responses that prevent hepatocarcinogenesis in the inflamed liver (Shalapour et al., 2017).

In addition, two studies suggest that activated B cells may directly induce tumor cell apoptosis (Garnelo et al., 2017; Jahrsdörfer et al., 2006).

Regulatory role

Tumor B cells produce antitumor antibodies of IgA isotype, as shown in NSCLC (Germain et al., 2014). These IgA-producing B cells were shown to modulate CD8T cells cytotoxicity in an HCC mouse model (Shalapour et al., 2017). Through production of immunosuppressive cytokines such as IL-10 (Shen and Fillatreau, 2015), B reg cells may locally inhibit T cell activation toward tumor-associated antigens.

Concluding remarks

In conclusion, B cells may be a double-edged sword, resulting either in tumor cell destruction by increasing T cell responses and via ADCC or in tumor growth by fueling chronic inflammation, angiogenesis, or immunosuppression via immune complex formation or complement activation. However, we still have a poor understanding of the heterogeneity and diversity of B cell subsets in tumors, which poses a major obstacle and dilemma when targeting B cells as oncological treatments. In addition, as for T cells, most intratumoral B cells may be bystanders and not antitumoral. The respective importance of bystander and cancer-specific B cells needs to be further evaluated in this emerging field.

The exploding investigations in this new field will not only provide a better understanding of antitumor immunity but also propose novel prognostic and predictive markers as well as novel therapeutic targets. Thus, the analysis of intratumoral

B cells by single-cell technology, the identification of their Ig repertoire, and the characterization of the antibodies they produce in long-survivor immunotherapy-responder patients will permit the design of new therapeutic monoclonal antibodies. We are at the very beginning of a newly open and very promising avenue.

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