Regulatory B cells in anti-tumor immunity

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Abstract
Advances in understanding of the immune microenvironment have highlighted the role of immunosuppressive T cell, myeloid, dendritic and monocytic sub-populations in inhibition of the anti-tumor immune response. The role of B cells in modulating the immune response to solid tumors as well as lymphoid malignancies is less well understood. Murine models of autoimmune disease have defined regulatory cell (Breg) subsets with immune suppressive activity, including B cell subsets that express IL-10, and transforming growth factor-β, which can facilitate T regulatory cell recruitment and expansion. Multiple murine tumor models point to the existence of similar immune suppressive B cell sub-populations that can migrate into tumor deposits and acquire an immune suppressive phenotype, which then leads to attenuation of the local anti-tumor immune response. Other murine models of viral or chemically induced skin carcinogenesis have identified a pivotal role for B cells in promoting inflammation and carcinogenesis. While many human solid tumors demonstrate significant B cell infiltration and/or tertiary lymphoid structure formation, the functional properties of tumor-infiltrating B cells and their effects on immunity are poorly understood. Recent successes in early Phase I/II trials using anti-checkpoint inhibitor antibodies such as nivolumab or pidilizumab directed against PD-1 in the setting of Hodgkin’s and non-Hodgkin’s lymphomas validate the therapeutic utility of reversing B cell-mediated immune suppression. Further studies to define Breg subsets, and mechanisms of suppression, may provide new avenues for modulation of the immune response and meaningful therapeutic intervention in both lymphoid and solid tumors.

Keywords: anti-tumor immunity, B regulatory cells, IL-10, TGF-β

Introduction
The tumor cell microenvironment plays a pivotal role in modulating immune response in the setting of cancer. Although the role of T regulatory cells (Tregs) and myeloid-derived suppressor cells as well as tumor-associated macrophages in shaping of the anti-tumor immune response has been well studied, an emerging role for B cells is being increasingly appreciated (1–9). In addition to Tregs, it appears that subsets of B cells with immunosuppressive and/or regulatory function may play a critical role in regulating immune responses to murine and human tumors, and/or may also participate in carcinogenesis (5–7, 10–14). In addition, in some murine models and human tumors, B cells may play a protective rather than immunosuppressive role (5, 7, 15–17). B cell subsets with distinct phenotype and function may play distinct roles in relation to anti-tumor responses.

B regulatory activity and autoimmunity
The concept of B regulatory cells (Bregs) was originally invoked in relation to autoimmune disease (18). Initial studies demonstrated that B cells may play a critical regulatory role in experimental autoimmune encephalomyelitis (EAE) and in suppression of intestinal inflammation in murine models (19, 20). Additional studies also demonstrated a convincing role for IL-10-producing B cells in regulating T cell responses in a model of collagen-induced arthritis (21). In these models, B cells producing IL-10 play a critical role, and in the absence of IL-10, autoimmune responses are exacerbated.

A broad range of B regulatory phenotypes have now been described that fulfill an immunosuppressive role in autoimmune diseases and cancer (Table 1). IL-10-producing Bregs (22, 23), tumor necrosis factor (TNF)-α-producing Bregs (11), T cell Ig domain and mucin domain 1 (TIM-1)+ Bregs (24) and IL-10-independent Breg cells have been described by several investigators (25,35,42). Fas-ligand (Fas-L)+ killer Bregs (43), Granzyme-B (GrB) Bregs (44), transforming growth factor (TGF)-β-secreting Bregs (34) or GITRL- Bregs (35) as well as IL-21, IL-33, IL-35 cytokine-driven Bregs (39, 40, 44) have also been described. More recently, CD73+ Bregs have been described that are capable of inducing adenosine production (36). Adenosine generation by B cells may lead to suppression of T cell...
**Table 1.** Breg subsets in human and mouse diseases models

<table>
<thead>
<tr>
<th>Types of Breg</th>
<th>Phenotype</th>
<th>Species</th>
<th>Diseases models</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10+ Breg</td>
<td>CD19-CD1d-CD5+</td>
<td>Human, mouse</td>
<td>EAE, CHS, RA</td>
<td>(19–22)</td>
</tr>
<tr>
<td>TIM-1 Breg</td>
<td>CD19-CD24+CD38hi</td>
<td>Human</td>
<td>SLE, RA</td>
<td>(21, 23)</td>
</tr>
<tr>
<td>iTNFR-α Breg</td>
<td>IL-10+</td>
<td>Mouse</td>
<td>Squamous carcinogenesis</td>
<td>(11)</td>
</tr>
<tr>
<td>LTα Breg</td>
<td>CD19-CD21+CD5-IL-10- IL-4-</td>
<td>Mouse</td>
<td>Islet allograft</td>
<td>(24)</td>
</tr>
<tr>
<td>Granzyme-B+ Breg</td>
<td>IL-21 induced: CD19-CD5+CD38+CD1d-CD1gM-CD1 +75+CD6+CD20-CD25-IL-10-IDO+GrB+</td>
<td>Human</td>
<td>Cancer (breast, ovarian, cervical, colorectal, prostate carcinomas)</td>
<td>(31, 32)</td>
</tr>
<tr>
<td>GITR+ Breg</td>
<td>CD19-TGF-β+TIM1+IL-10-CCR6+CXCR3+</td>
<td>Human</td>
<td>HIV-1</td>
<td>(32)</td>
</tr>
<tr>
<td>CD63+ Breg</td>
<td>UP-regulate pro-angiogenic genes, such as, VEGF, IF1α, MMP9, MMP2, CCL2</td>
<td>Mouse</td>
<td>Islet transplant</td>
<td>(34)</td>
</tr>
<tr>
<td>STAT3+ Breg</td>
<td>UP-regulate pro-angiogenic genes, such as VEGF, MMP9, IF1α</td>
<td>Human</td>
<td>Melanoma, prostate cancer</td>
<td>(37, 38)</td>
</tr>
<tr>
<td>LTα Breg</td>
<td>CD19-CD21+CD23+CD24+CD1d+ IL-10+</td>
<td>Human, mouse</td>
<td>Castration-resistant prostate cancer</td>
<td>(14)</td>
</tr>
</tbody>
</table>

**Cytokine-driven Breg induction**

| IL-21 | CD19-CD5+CD38+CD1d+CD1gM-CD147+CD86+CD154+CD20-CD25+CD10-IDO+GrB | Human, mouse | Normal, HIV, MS | (31, 32) |
| IL-33 | CD19-CD25+CD1d+CD5-CD63-CD23-Tim-1-IL-10- | Mouse | Enterocolitis | (39) |
| IL-35 | CD19-CD220+CD5-CD1d- produce IL-35, IL-10 | Mouse | EAU | (40) |
| IL-6, IL-1β | CD19-CD21+CD23+CD24+CD1d+ IL-10+ | Mouse | Arthritis, inflammation | (41) |

CHS, contact hypersensitivity response; CLL, chronic lymphocytic leukemia; EAU, experimental autoimmune uveitis; HIV, male specific histocompatibility antigen; MM, myeloid myeloma; MRL, Murphy Roths Large mice; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

activation and/or anergy. In other models of murine arthritis, microbiota in the gut promote macrophage-mediated secretion of IL-6 and IL-1β which in turn promote differentiation of Bregs which produce IL-10 and suppress local inflammation (41). IL-33-induced Breg cells which also produce IL-10 have been described which attenuate mucosal autoimmune inflammatory responses (39). IL-35 is a member of the IL-12 family of cytokines that may induce differentiation of Treg cells. Wang et al. demonstrated that IL-35 can induce B cell differentiation into Bregs (40), and such Bregs in turn produce additional IL-35 and IL-10 and suppress autoimmunity in models of autoimmune uveitis in vivo.

The TIM proteins are a family of co-stimulatory molecules that play an important role in differentiation of CD4+ effector cells. TIM-1 is expressed on activated CD4+ cells and polarized T2 cells in vitro and also expressed on a subset of B cells. TIM-1+ B cells produce significant amounts of IL-4 and IL-10 upon TIM-1 ligation (24). In mice, TIM-1 is expressed on IL-10-expressing regulatory B cell sub-populations, including transitional, marginal zone and follicular B cells, as well as in the CD1d+ GrB+ CD5+ Breg population. A role for TIM-1+ B cells in human tumors has not yet been described. However, a variety of B cell malignancies derive from phenotypically similar B cell subsets and are likely to have similar regulatory properties.

Fas-L+ Bregs have been described in allergy and autoimmunity as well as in transplantation tolerance (30, 46, 47). Fas-L positivity has been described in the setting of non-Hodgkin's lymphoma (NHL), diffuse large B cell lymphoma (DLBCL) and Burkitt's lymphoma, and in freshly isolated B cells from human lymphoid organs (26–29, 48). Fas-L+ B NHL cell lines promote cell death when cultured with susceptible T cell lines (27). Killing of T cells by B chronic lymphocytic leukemia cells can be blocked using neutralizing antibodies against either Fas or Fas-L (27). Activated B cells may express Fas-L and can mediate cell death (40–41). Expression of Fas-L and IL-10 appears to be highest in CD5- B cell populations suggesting that the CD5- cells may have a specialized B regulatory function (43). A role for Fas-L+ Bregs has not yet been clearly demonstrated in the setting of non-lymphoid tumors and requires additional investigation. In another study, a granulocyte macrophage colony-stimulating factor/IL-15 fusokine was capable of inducing B cells with suppressive function. Fusokine-induced B cells expressed MHC-I, MHC-II, surface IgM and IgD, could secrete IL-10 and were capable of inhibiting EAE in murine models (45). The fusokine-induced immune suppressive function appears to be mediated through JAK-STAT signaling (45).

GrB is a cytotoxic protease, has a known role in cytolytic activity, and is also expressed in Tregs as well as plasmacytoid dendritic cells (52, 53). Human Gr-B+ cells have been described (GrB+ Bregs) (44). GrB+ Bregs have been found within the microenvironment of multiple human tumor types including breast, ovarian, cervical, colorectal and prostate carcinomas (44). B cells that express GrB have been found adjacent to IL-21-secreting Tregs that contribute to immune tolerance (44). IL-21 has been shown to induce the outgrowth of B cells expressing high levels of GrB, which can
inhibit T-cell proliferation through GrB-dependent degradation of the T-cell receptor (TCR)ζ chain. GrB+ B cells express key regulatory molecules implicated in immune tolerance, including IL-10, CD25 and indoleamine-2, 3-dioxygenase. IL-21 may induce GrB+ human Bregs that may infiltrate solid tumors and suppress anti-tumor immune responses, while others have demonstrated that IL-21 can actually induce expression of GrB in B cells, which may actually be cytotoxic to tumor cells (31). Whether GrB+ B cells isolated from tumor tissues are actually immunosuppressive is the subject of controversy. IL-21 appears to induce Breg differentiation in several settings. CD4+CD40+ T cells from HIV-1+ patients can produce IL-21, that in turn promotes B cell differentiation into GrB+ Bregs in vitro, which express CD5, CD43, CD86 and CD147, but not IL-10. Such IL-21-invoked GrB+ B cells can degrade the TCR-ζ chain and suppress T cell proliferation (32). Similarly, in kidney transplant patients, IL-21 appears to potentiate development of GrB+ Bregs, which can in turn reciprocally stimulate T cell IL-21 secretion, implicating a positive feedback loop that leads to immune tolerance (33). In the presence of IL-21 and CD40 signaling, murine B cells can differentiate into B10 Bregs, which demonstrate exceptionally high levels of IL-10 secretion compared with normal B cells, and suppress multiple sclerosis development in a murine model (54).

The plethora of B cell subtypes with professed regulatory function suggest that B cells may act to stimulate or alternatively to suppress anti-tumor immune response. Careful phenotyping of tumor-infiltrating B cell populations may disclose sub-populations that may be playing an important role in modulating anti-tumor responses. A diverse array of phenotypically distinct Bregs has been implicated in autoimmune models, and it is reasonable to assume that analogous B cell subsets will be identified in association with malignancy.

**B regulatory activity in cancer models**

A role for B cells in anti-tumor immunity was demonstrated by Brodt and Gordon (9) and Monach et al. (8) and later by Qin et al. (1) and Shah et al. (2). Augmented anti-tumor immunity was noted in the absence of B cells, and the immune response was reduced when B cells were infused into B cell-deficient mice (BCDM) (2, 42). In the MC38 murine colon cancer model, tumors grew slowly or completely regressed in BCDM but grew briskly following adoptive transfer of normal B cells. The tumor bed in BCDM was heavily infiltrated with CD4+ and CD8+ T cells, in comparison to wild type (WT) mice. MC38 tumor rejection in BCDM correlated with an increased T1, response and increased levels of CD8+ T cell cytotoxicity directed against tumor cells. Transfer of B cells into BCDM restored MC38 growth (2, 42).

A variety of murine tumors were subsequently noted to grow more efficiently in WT mice as opposed to BCDM. These included EMT-6 mammary carcinoma, EL-4 thymoma and B16.F10 melanoma (2, 42, 55). EL-4 tumor growth was markedly reduced in BCDM relative to WT mice. However, EL-4 tumors grew normally in B cell receptor (BCR) deficient transgenic MD4 B cells with BCR specificity for an epitope on hen egg lysozyme (2). Thus, it appeared that B cell facilitation of tumor growth was occurring through antigen

non-specific interactions with T cells. In each of these models, T1, responses as manifest by induction of CD8+ IFN-γ-secreting cells and CTL activity were enhanced in BCDM compared with WT mice.

The role of MHC-II molecules was also tested in the EMT-6 model (56). While implanted EMT-6 showed diminished growth in BCDM, adoptive transfer of either MHC-II−/− B cells or WT B cells into BCDM rescued tumor growth. This result suggested that direct antigen recognition or presentation by B cells did not appear to be critical in conferring tolerance.

Consistent with a B cell immunosuppressive effect, enhanced activity of melanoma vaccines was observed in the absence of B cells (57). Similarly, vaccination against the EG-7 tumor model (EL-4 cells transduced to express ovalbumin) using EG-7 cells expressing secreted gp-96Ig, was more effective in BCDM mice compared with normal mice. Using gp-96Ig-mediated vaccination against EG-7, more effective tumor protection was achieved in BCDM (58).

**Breg effects on T1,2 differentiation and Treg expansion and function**

B cells serve as antigen-presenting cells that are important in CD4+ T cell activation, proliferation and differentiation. In addition to cytokine secretion by the T cells themselves, a variety of cytokine-producing B cell subsets have been described. IFN-γ-producing B cells (Be1) have been noted to promote naive CD4+ T cell differentiation into T1, cells, while IL-4-producing B cells, so called Be2 cells, can facilitate differentiation of CD4+ naive T cells into T2 cells (59). Whether Be1 or Be2 cells play a critical role in anti-tumor responses is unknown.

Another subset of T cells, CD4+CD25+FoxP3+ Tregs, play an important role in promoting tumor tolerance and minimizing host anti-tumor responses (60, 61). In murine models of autoimmune colitis, B cells were noted to facilitate conversion of naive T cells into Tregs (62). Similar effects on Treg development and expansion are seen when B cells facilitate tumor growth. Following EMT-6 mammary tumor implantation, increased Treg infiltration and expansion were noted in WT mice in comparison to BCDM. The percentage and absolute number of Tregs found in spleen, tumor-draining lymph nodes and within the tumor bed were reduced in tumor-bearing BCDM compared to WT mice (55). Treg inhibitory function, as assayed using T cell proliferation suppression assays, was reduced in Tregs from tumor-bearing BCDM, compared with WT mice, suggesting a qualitative defect, as well as reduction in Treg number (55). Depletion of Tregs using an anti-CD25 antibody (PC61) restored anti-EMT-6 immunity, implicating Tregs as key mediators of B cell effects on tumor growth (42).

In another murine breast cancer model, 4T1, Olkhanud et al. demonstrated that tumor growth and metastatic spread to the lung are dependent on the presence of tumor evoked Bregs (tBregs) (63). The tBreg-related support of tumor growth appeared to be mediated through conversion of resting CD4+ T cells into Tregs (63). Specifically, a CD19+ B220+CD25+ B cell subset was able to induce conversion of naive CD4+ T cells into Tregs in vitro and/or in vivo. Conversion of CD4+ T cells appeared to be contingent on cell to cell contact between T and B cells and on TGF-β secretion by tBregs.
Breg activity and the role of IL-10, CD40 and OX40L

IL-10 produced by B cells has been demonstrated to play a significant role in modulating the severity of autoimmune diseases such as EAE (64). BCDM on a C57BL/6 background develop a severe non-remitting form of EAE following immunization with myelin oligodendrocyte glycoprotein. Recovery from EAE is associated with production of IL-4 and IL-10.

IL-4−/− mice develop EAE and eventually enter remission, whereas IL-10−/− mice, demonstrate a non-remitting EAE similar to BCDM (20). Yanaba et al. have further defined a CD19+CD1d+regulatory CD5+ Breg population (B10 regulatory cells) that suppresses the inflammatory response in EAE through release of IL-10 (22). Corresponding IL-10-producing B cell subsets have been identified by lwata et al. in humans (65).

In contrast, in a murine EAE model induced by myelin basic protein-reactive T cells generated in vitro, Ray et al. demonstrated that B cell-mediated expansion of Tregs depended on interactions of the glucocorticoid-induced TNFR (GITR) and its ligand but was not dependent on IL-10 (35).

In another murine model using DO11.10 OVA-TCR transgenic mice, so-called B-1a Bregs are able to induce conversion of CD4+CD25+ T cells into a subset of T cells with suppressive function (66). These induced 'Treg-of B1a' immunosuppressive T cells up-regulate OX40, PD-1, ICOS and IL-10R but do not express FoxP3. Interestingly, both IL-10-producing and IL-10−/− B-1a cells can support conversion of CD4+CD25+ T cells into Tregs in vitro. Induction appears to be primarily dependent on CD86-mediated co-stimulation, rather than IL-10. Treg-of-B1a cell-mediated suppression through an IL-10- and TGF-β-independent pathway, representing a distinct Treg population from FoxP3+ Tregs (66).

Similarly, in the EMT-6 model, adoptive transfer of B cells from either WT or IL-10−/− mice was capable of restoring tumor growth in BCDM. Significant NK cell and CD8+ T cell infiltration was noted in BCDM, but NK and CD8+ infiltration were markedly diminished in either WT or IL-10−/− B cell reconstituted BCDM. Both IL-10−/− B cells and WT B cells facilitated Treg proliferation in vitro in mixed leukocyte reaction assays. IL-10 secretion by B cells was not critically required for B cell-mediated suppression of anti-tumor immunity in the EMT-6 model (42).

OX40L expression on B cells is required for optimal CD4+ T cell clonal expansion and memory cell development (67). OX40 receptor -OX40L engagement promotes T,2 responses and cytokine production in vivo and in vitro (67–70). The role of OX40L in modulating anti-tumor immunity was tested in the MC38 model. MC38 tumors grew progressively in WT mice and in OX40L−/− mice, but tumor growth was inhibited in BCDM. BCDM reconstituted with WT B cells supported tumor growth. In contrast, tumor growth was not supported in BCDM reconstituted with OX40L−/− B cells. Engagement of OX40 on T cells, by OX40L-expressing B cells, may suppress anti-tumor response, in part through inhibition of IFN-γ production and CTL generation (56). In contrast to OX40L, when MC38 tumors were implanted in BCDM reconstituted with either WT B cell or CD40−/− B cells, MC38 tumors grew progressively, although a minor delay in growth was noted in CD40−/− B cell reconstituted BCDM. Therefore, CD40−/− B cells were able to rescue anti-tumor responses in vivo despite decreased efficiency in inhibiting IFN-γ secretion in splenocyte B cell co-cultures in vitro.

Tumor cell ‘education’ of Bregs

EMT-6 tumors in WT mice are heavily infiltrated with B cells (TIL-Bs). CD19+ TIL-B cells isolated from tumors are considerably more suppressive of CD4+ T cell proliferation in vitro than B cells isolated from the spleens of either tumor-bearing or non-tumor-bearing animals. This indicates a local change in the character of B cells conferring an immunosuppressive phenotype. Co-cultivation of normal B cells with the EMT-6 tumor cells in vitro also results in acquisition of a suppressor phenotype. In vitro generated Bregs exposed to EMT6 tumor cells suppressed proliferation of CD4+CD25− T cells, and suppression appears to require direct B–T cell contact. A variety of immune suppressive ligands are expressed on TIL-Bs. The TIL-Bs demonstrate enhanced expression of PD-L1, CD86, IAδ and LAP/TGF-β. Both PD-L1 and TGF-β appear to play a role in promoting EMT-6 tumor growth since blocking of either using antibody results in decreased tumor growth. Increased expression of LAP/TGF-β on the B cell surface is acquired following migration of B cells into the tumor bed, where they undergo phenotypic conversion into Bregs (Fig. 1, Zhang et al. unpublished results).

Olkhanad et al. (63) described a subset of B cells in the 4T1 mouse breast tumor model resembling mature B2 cells (CD19+CD25−/CD69+) that constitutively express activated Stat3 and display B7-H1, CD81, CD86, CD62L and IgM. These B2 cells facilitated the establishment of lung metastasis in part through TGF-β-dependent conversion of FoxP3+ Tregs from CD4+ T cells (17). Another study of the 4T1 breast cancer model suggested that tumor-produced metabolites of 5-lipoxygenase promote development of a B regulatory phenotype through effects on the peroxisome proliferator-activated receptor α (PPARα) receptor (71). Metabolites such as leukotriene B4, appear to activate PPARα in B cells. PPARα activation appears to be critical to the development of Treg function.

Other factors have also been implicated in B cell differentiation into regulatory B cells. Placental growth factor (PIGF) is a member of the vascular endothelial growth factor subfamily. This factor has been implicated in angiogenesis and placental growth. Glioma cells have been noted to secrete exosomes containing high levels of PIGF mRNA and protein (72). When such PIGF-carrying exosomes are co-cultured with B cells, they appear to promote differentiation into TGF-β+ B cells (72). Intratumoral B cells were noted to specifically proliferate in response to glial-related antigens. In this model, glial cell-released PIGF appears to induce tumor-infiltrating B cells to become TGF-β+ Bregs that suppress CD8+ responses (72). Thus, in human gliomas, PIGF may play a role in mediating conversion of B cells into a regulatory phenotype.

In addition to modulating anti-tumor immunity, B cells have been shown to play a role in promoting tumor angiogenesis (37, 38). B cells with or without activated Stat3 have opposite effects on tumor growth and tumor angiogenesis in the B16 melanoma and Lewis Lung Carcinoma mouse models. B cells with activated Stat3 are found in proximity to tumor...
vasculature and the density of B cells in human tumor tissues correlated with the expression levels of Stat3-induced proangiogenic genes and with the extent of tumor angiogenesis (37). Many human tumors are heavily infiltrated with B cells including non-small-cell lung carcinoma, head and neck, breast and/or ovarian cancer. The potential immunosuppressive and/or angiogenic properties of these B cells have not been adequately studied.

A B cell role in carcinogenesis

B cells have also been implicated in carcinogenesis in a skin carcinoma model (10). Using a K14-HPV16 transgenic mouse model of inflammation-associated epithelial carcinogenesis, de Visser et al. reported that elimination of mature T and B lymphocytes limited neoplastic progression. Adoptive transfer of B lymphocytes or serum from HPV16 mice into T and B cell-deficient/HPV16 mice restored innate immune cell infiltration, increased chronic inflammation and led to a hyper-proliferative epidermis. B lymphocytes were required for establishment of a chronic inflammatory state that promoted carcinogenesis (10).

A role for B cell auto-antibodies in promoting inflammation has also been proposed, through activation of Fcy receptors on recruited myeloid cells (73). Stromal accumulation of auto-antibodies in premalignant skin appears to regulate recruitment and composition of leukocytes in neoplastic tissue in a manner that promotes carcinoma development. The nature of such B cell–myeloid interactions is still unclear.

In a chemically induced skin carcinoma model (DMBA/TPA-induced squamous carcinoma), tumor development was significantly reduced in RAG2−/− mice but could be induced by adoptive transfer of WT B cells, but not TNF-α−/− B cells. These results implicated TNF-α secretion by B cells in pathogenesis (11). Resistance to papilloma development in TNF-α−/− mice was associated with increased IFN-γ+CD8+ T cell infiltration in skin and spleen and a reduction in IL-10-producing Bregs. In this model, TNF-α appeared to promote tumor development via Bregs that repress anti-tumor immunity.

Using the K14-HPV16 mouse model, depletion of B cells using an anti-CD20 mAb improved the therapeutic response to cisplatinum- and taxol-based chemotherapy. An improved response appeared to be induced through an increase in CD11b+Gr1+CD4+CD20+CD11c− macrophages and CD8+ T cell infiltration into tumors, and a decrease in recruitment of CD11b+Gr1+CD4+CD11c− immature myeloid cells, mast cells and Gr1+ cells (74). The improved response to chemotherapy depended on enhanced infiltration of tumors with activated CD8+ lymphocytes through a CCR5-dependent mechanism. Analysis of CD11b+Gr1+CD11c− macrophages isolated from squamous cell carcinomas of anti-CD20/cisplatinum/taxol-treated mice versus control mice demonstrated increased mRNA expression of several chemokines implicated in leukocyte recruitment including CCL2, CCL3, CCL5, CCL7 and CCL9. Macrophages that were isolated from B cell depleted mice appear to be skewed to the M1 phenotype (74). In this model, the presence or absence of B cells affected the nature of macrophage infiltration in tumors, in turn influencing response to chemotherapy.

Malignant B cells and modulation of anti-tumor responses

Malignant B cells in lymphoma evoke normal mechanisms of interaction in shaping the tumor microenvironment and thereby modulating anti-tumor immune responses (75). Malignant B non-Hodgkin’s lymphomas do not represent homogeneous populations of malignant B lymphoid cells but rather contain abundant populations of non-malignant cells which are attracted to the tumor microenvironment that shape the local immune response (76). For example, in follicular lymphoma, a significant percentage of intratumoral lymphoid...
cells may not derive from the malignant clone, and appreciable numbers of infiltrating CD3+ T cells can be seen (77). Several studies suggest that infiltration with CD4+ T cells and/or CD8+ T cells in malignant lymphoma may correlate with improved survival, yet the overall immune response appears to be ineffective, and immune elimination of malignant lymphomas is generally not observed (78, 79). Possible exceptions may include the disappearance of malignant gastric lymphomas following eradication of Helicobacter pylori (80), anecdotal responses of marginal zone lymphomas to antiviral therapy (81) and the frequent regression of post-transplant lymphoproliferative disorder observed following withdrawal of immunosuppression (82).

Various mechanisms that can impair an anti-lymphoma immune response have been invoked, including the presence of high numbers of Tregs, expression of immunosuppressive co-inhibitory ligands such as PDL-1/2 by lymphoma cells, the induction of T cell exhaustion and the recruitment of immunosuppressive cell populations such as monocytes and myeloid-derived suppressor cells into the tumor bed (83, 84). Studies by Yang et al. and Ansell (79, 85) have demonstrated the ability of malignant B lymphoma cells to induce expansion of FoxP3+ Tregs. The Tregs may be recruited to the tumor bed through the local elaboration of chemokines such as CCL22, which may interact with the CCR4 chemokine receptor to recruit Tregs (78, 79). Furthermore, malignant B lymphoid cells may increase recruitment of Tregs while suppressing the development of effector populations such as T\textsubscript{1}7 lymphocytes (86). Other studies have also demonstrated evidence of T cell exhaustion, as manifest by a TIM-3-CD3+CD8+ T cell immunophenotype in lymphoma samples, thought in part due to local elaboration of cytokines such as IL-12 (87).

Malignant B lymphoma cells may also directly express a variety of suppressive ligands and cytokines (85). Examples include the high levels of expression of membrane-bound TGF-β on lymphoma B cells (88). B cells may produce TGF-β, which can in turn induce CD70 expression on effector T cells leading to T cell exhaustion (89). TGF-β may affect the response in both soluble and membrane-bound forms. TGF-β produced by malignant B cells and by intratumoral T cells can promote development of the Treg population and inhibit T\textsubscript{1}- and T\textsubscript{1}7-mediated responses (86). A compelling example of B regulatory activity involves expression of immune inhibitory ligands by the malignant Reed Sternberg cells in the setting of classic Hodgkin’s lymphoma (90). PD-1 is a regulatory receptor which functions as a check point inhibitor of the immune response. Two ligands for PD-1 have been described, PD-L1 is broadly distributed across a variety of tissues and PD-L2 is limited principally to antigen-presenting cells (91). Interactions between PD-1 on T lymphocytes and PD-L1 and PD-L2 have been linked to the generation of immune tolerance and directly promote immune survival of malignant Reed Sternberg cells (90). The PD-1 ligand genes PD-L1 and PD-L2 are located on chromosome 9p24.1, an area in which copy gain has been described in association with nodular sclerosing Hodgkin disease (92). The 9p24.1 amplification region includes the PD-L1 and PD-L2 ligands, as well as the JAK2 locus (93). Immunohistochemical analysis of Reed Sternberg cells demonstrates frequent positivity for PD-L1. PD-L1 expression also appears to be induced by EBV infection and/or Major Histocompatibility Complex Class II Transactivator (CIITA) translocations (94). Expression of PD-1 ligands by Reed Sternberg cells provided a rationale for targeted blockade of PD-1. In a study by Ansell et al. in patients with relapsed and/or refractory Hodgkin’s disease, PD-1 blockade with nivolumab was found to be highly effective, resulting in an 87% overall response rate, with durable responses seen in many patients who had failed salvage therapy, including brentuximab and/or stem cell transplant (95). Similar results have been seen with another anti-PD-1 antibody pembrolizumab as reported by Moskowitz et al. at the 2014 ASH meetings. Of 10 enrolled patients whose samples were studied and evaluable for PDL-1 expression, 100% were PDL-1+.

Tumor-infiltrating B cells in human tumors

A variety of mouse models differ with the respect to the effect of B cells on tumor growth. Tumorigenesis by HPV16 in the skin appears to be reduced in the absence of B cells, and the growth of diverse tumors such as EL-4 lymphoma, MC38 carcinoma, D-5 melanoma and the EMT-6 and 4T1 mammary carcinomas all are reduced in murine models, suggesting roles for B cells in carcinogenesis and/or in the attenuation of anti-tumor immune response. This has led to the realization that similar B cell effects may be operative in human solid tumors. However, results in the human setting are limited and conflicting.

Tumor-infiltrating B cells have been studied in a variety of settings. In breast cancer, these are reportedly present in a subset of tumors and often comprise a substantial percentage of the tumor-infiltrating lymphocyte population (12). Often, TIL-Bs may be found in complex aggregates of B cells, T cells and dendritic cells with variable architecture known as tertiary lymphoid structures (TLS) (98, 99). TLS can be found at the sites of inflammation, and in association with cancer in various organs and participate in local immune responses (98, 99). TLS have been described in association with medullary carcinoma of the breast as well as in ovarian cancer (100–103). TIL-Bs have been associated with improved survival in the setting of medullary breast cancer (100–103). In the setting of non-small-cell lung cancer, the presence of CD8+, CD4+ and CD20+ TILs has been linked to improved
survival (104–107). Similar findings have also been implicated in cervical cancer (108). TIL-Bs are frequently found in serous ovarian cancers in association with CD4+ and CD8+ T cells and appear to correlate with improved survival as well (109). The role played by infiltrating B cells in TLS is poorly understood, and it is not known whether B cells within TLS serve to facilitate or dampen local response.

An infiltrating B cell signature (Ig and CD20 mRNA) has recently been identified frequently in patients with head and neck squamous cell carcinoma, as well as pancreatic ductal adenocarcinoma (PDAC) and may portend an adverse prognosis, leading to planned trials combining chemotherapy with gemcitabine and ibrutinib, a BTK inhibitor in PDAC (Coussens LM, Plenary session, AACR, 2015, Philadelphia).

It also appears that discrete tumor-associated antigens may occasionally be recognized by B cells. A wide variety of humoral immune responses against tumor-related antigens have been reported (110). Generally such responses are not protective. Multiple studies provide evidence for local antigen-driven expansion and affinity maturation consistent with TIL-B responses to local antigens. In medullary breast cancer, TIL-B-derived antibodies that recognize a ganglioside D3 and β-actin have been described (111–113). Mutated PS3 appears to be recognized by TIL-B antibodies in lung cancer and in colon carcinoma (114, 115). The relationship between auto-antibody production and clinical outcome is unclear. In general, such auto-antibodies are not protective but nevertheless indicate engagement of tumor antigens by local B cell populations.

Conclusions

Extensive evidence in animal models suggests that tumor-infiltrating B cells are not innocent bystanders, and exert profound effects on anti-tumor immunity. It is interesting to speculate whether the presence of B cells, despite association with improved prognosis in some malignancies, may actually mask an immune response that might be substantially more effective in the absence of such infiltrating B cells. In this regard, the careful characterization of the phenotype and/or suppressive properties of tumor-infiltrating B cells has not been performed in the setting of human solid tumors. Immune anti-tumor responses may be enhanced or alternatively suppressed depending on the B cell subsets that are recruited to the tumor site. As B regulatory subsets associated with malignancy are better characterized in animal models, this may facilitate the search for corresponding subsets in human tumors.

Careful identification of B regulatory subsets and their immune inhibitory properties may allow selective depletion of inhibitory B cells using anti-B cell antibodies or more recently B cell-specific agents such as Bruton’s tyrosine kinase inhibitors and/or other signal transduction inhibitors targeting the BCR signaling pathway. Cross-talk between B, T, and NK lymphocytes as well as tumor-associated macrophages, needs to be better understood in order to characterize the potential role of B cells in modulating anti-tumor immunity, in recruitment and/or expansion of the Treg sub-population within tumors, as mediators of carcinogenesis, and/or as anti-tumor effector cells. Potential effects of B cell depletion in the setting of non-lymphoid tumors are poorly understood and evidence from animal models that depletion of B cells will facilitate responses is lacking. As an example, depletion of B cells using rituximab in the setting of IL-2 therapy for melanoma and renal cell carcinoma did not appear to substantially alter response (116) Whether Breg subsets are effectively depleted using anti-CD20 antibodies is unknown. Nevertheless, the availability of agents that can selectively deplete B cells including anti-CD20 antibodies (rituximab, ofatumumab, obinutuzumab, etc.), and/or BTK, Lyn, PI3K-δ and Syk inhibitors, and newer antibodies directed at other targets such as CD22 and CD19, all may provide means by which to reverse effects of B cell infiltration and attenuate B cell effects on anti-tumor immune responses. Whether selective B cell depletion can be combined with other effective means of enhancing anti-tumor immunity such as the use of checkpoint inhibition, to improve anti-tumor immune response, will likely be the target of further study.

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