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CD20+ B Cells: The Other Tumor-Infiltrating Lymphocytes

Brad H. Nelson

Tumor-infiltrating CD8+ T cells are strongly associated with patient survival in a wide variety of human cancers. Less is known about tumor-infiltrating CD20+ B cells, which often colocalize with T cells, sometimes forming organized lymphoid structures. In autoimmunity and organ transplantation, T cells and B cells collaborate to generate potent, unrelenting immune responses that can result in extensive tissue damage and organ rejection. In these settings, B cells enhance T cell responses by producing Abs, stimulatory cytokines, and chemokines, serving as local APCs, and organizing the formation of tertiary lymphoid structures that sustain long-term immunity. Thus, B cells are an important component of immunological circuits associated with persistent, rampant tissue destruction. Engagement of tumor-reactive B cells may be an important condition for generating potent, long-term T cell responses against cancer. The Journal of Immunology, 2010, 185: 4977–4982.

The field of tumor immunology is strongly focused on CD8+ T cells, owing to their ability to directly kill tumor cells and the strong association between tumor-infiltrating CD8+ T cells and patient survival in many cancers (1). In contrast, B cells are often overlooked by tumor immunologists, likely because of the common notion that humoral and cytolytic responses work in opposition. Yet, B cells figure prominently in the fields of autoimmunity and tissue transplantation, settings in which T cell responses are so strong and persistent as to override the best attempts at immunosuppression. Given that the immune response to cancer develops over many years and, one hopes, can be manipulated to provide protection on a time scale of decades, there is undoubtedly much to be learned from the chronic immune responses seen in autoimmunity and transplantation. In this study, I will compare B cell responses in cancer, autoimmunity, and transplantation, with the goal of elucidating the mechanisms used by B cells to facilitate long-term T cell responses.

B cell development and differentiation

Human B cells develop in the bone marrow and initially have a naive phenotype manifested by unmutated Ig status, expression of IgM and IgD, and a CD27−CD38− surface phenotype (2). After activation by Ag, B cells enter primary follicles of lymph nodes or other lymphoid tissues where they undergo extensive proliferation, forming germinal centers (GCs) in which somatic hypermutation and class switching to IgG, IgA, or IgE take place. In the GCs, B cells receive growth and differentiation signals from follicular Th cells and compete for Ags presented by follicular dendritic cells (FDCs) in a process known as affinity maturation. B cells further differentiate into plasma cells (which produce high-affinity Abs) and long-lived CD27+CD38− memory cells (which respond to subsequent Ag encounters). CD20 is expressed on all mature B cells except plasma cells (3).

Although B cells typically reside in conventional lymphoid tissues such as spleen, lymph node, or blood, they can also be found in nonlymphoid tissues in aggregates with other immune cells. Such aggregates have been given various names but will be referred to here as tertiary lymphoid structures (TLSs). TLSs range from small aggregates of B cells, T cells, and DCs through to highly organized structures containing GCs, FDCs, T cell zones, high endothelial venules, and lymphatic vessels (4, 5). TLSs are found at sites of infection or inflammation in essentially any organ of the body and facilitate rapid and robust local immune responses (4, 5). As discussed later, TLSs are also seen in chronic immune responses associated with autoimmunity, allograft rejection, and cancer.

B cells in autoimmunity

In addition to their important role in immunity to pathogens, B cells contribute to various human autoimmune diseases through Ab-dependent and -independent mechanisms (6, 7). Autoantibodies contribute to autoimmune pathogenesis by inhibiting the function of their target proteins, activating the complement system, augmenting Ag presentation by DCs, or causing end-organ damage through the formation of immune complexes. In addition to making Abs, autoreactive B cells

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Abbreviations used in this paper: AR, acute rejection; DC, dendritic cell; FDC, follicular dendritic cell; GC, germinal center; RA, rheumatoid arthritis; TIL, tumor-infiltrating lymphocytes; TIL-B, tumor-infiltrating B cell; TLS, tertiary lymphoid structure; Treg, regulatory T cell.

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can enhance T cell responses through Ag presentation, co-stimulation, and modulation of DC migration and function (6, 7). For example, in the MRL/Mp-\textit{jpr/lpr} model of lupus, mice that are genetically deficient in B cells not only fail to develop autoantibodies but also show greatly attenuated CD4+ and CD8+ T cell activation and reduced lymphocytic infiltration of end organs (8, 9).

Auto-reactive B cells are found in TLSs in many autoimmune conditions, including rheumatoid arthritis (RA), SJögren’s syndrome, multiple sclerosis, autoimmune thyroiditis, diabetes, and lupus nephritis (4–6). In RA, TLSs are located in synovial tissue and serve as sites of clonal expansion, affinity maturation, and autoantibody production by B cells (7, 10–12). In a xenograft model in which affected synovial tissue from patients with RA was implanted in \textit{scid} mice, depletion of synovial B cells lead to the disappearance of TLSs, reduced activation of T cells, and reduced levels of TNF-\(\alpha\) and IFN-\(\gamma\) (13). Similarly, depletion of CD8+ T cells resulted in TLS disintegration and decreased lymphotixin and Ig secretion (14). Thus, B cells and T cells collaborate to maintain TLS structure and function.

The anti-CD20 mAb rituximab has been used to deplete B cells in various autoimmune conditions (6, 7). In RA, rituximab reduces autoantibodies as expected, yet it also benefits many autoantibody-negative patients by disrupting Ab-independent functions of B cells (7, 15). Specifically, rituximab causes the disappearance of TLSs, with concomitant loss of plasma cells, T cells, macrophages, and FDCs (16). Likewise, in patients with lupus, rituximab treatment results in decreased T cell activation and increased circulating regulatory T cells (Tregs) (15). In idiopathic thrombocytopenic purpura, rituximab can restore a normal CD4+ Th1/Th2 ratio (17). Finally, in multiple sclerosis, rituximab reduces the number of B and T cells in cerebral spinal fluid, with associated clinical benefit (18). Collectively, the use of rituximab in autoimmune reveals a central role for B cells in the maintenance of pathological T cell responses.

B cells in allograft rejection

B cells also play a major role in allograft rejection. In a landmark study, renal allograft biopsies from patients undergoing acute rejection (AR) were subjected to gene expression profiling (19). As expected, there was a strong gene signature associated with T cells, NK cells, and macrophages. Unexpectedly, a B cell signature (including CD20, CD74, and Ig) was also prominent. By immunohistochemistry, \(\sim 40\%\) of AR samples showed aggregates containing B cells, CD4+ T cells, CD8+ T cells, and macrophages (20). These aggregates were not associated with Ig or complement deposition, suggesting an Ab-independent role for B cells (19, 20). The presence of CD20+ B cells correlated with glucocorticoid resistance and graft loss. B cell infiltrates have also been described in human liver transplants undergoing AR (21), as well as cardiac transplants (22, 23).

B cells are thought to promote graft rejection by three major mechanisms (24). First, they produce donor-reactive Abs, which can damage tissue via complement and Ab-dependent cytotoxicity (23). Second, B cells produce cytokines and chemokines that can directly damage grafts as well as recruit T cells. Third, B cells can serve as APCs (25). For example, in a mouse cardiac allograft model, B cells contributed to graft rejection by presenting alloantigens to CD4+ T cells (26).

Depletion of CD20+ B cells with rituximab can ameliorate renal allograft rejection (27–31). Not only does rituximab reduce CD20+ B cell aggregates in kidney tissue, it reduces expression of T cell-associated gene products such as OX40, Fas ligand, and granzyme B (29, 30). Thus, as in autoimmune, B cells appear to facilitate pathological T cell responses against tissue allografts.

B cells in cancer

In contrast to the above findings in autoimmunity and transplantation, initial studies in mouse tumor models suggested that B cells generally inhibit T cell responses. For example, studies comparing wild-type and B cell-deficient mice found that B cells inhibit T cell-mediated regression of established tumors (32, 33), as well as T cell responses to cancer vaccines (34–36). B cells can impair the priming of CD8+ CTL responses by CD4+ T cells and instead promote non-protective humoral immune responses (37). Notably, however, other murine tumor studies have shown positive effects of B cells on T cell responses (38, 39). How can these conflicting results be reconciled? A key factor may be the activation status of B cells in different contexts, as T cell responses appear to be inhibited by resting B cells but facilitated by activated B cells (25, 40). Thus, studies in B cell-deficient mice, which lack both resting and activated B cells, could potentially yield conflicting results depending on the extent of B cell activation in the particular model system. As described below, B cells are commonly activated in human cancer patients, raising the possibility they play a positive role in tumor immunity.

Since the advent of serological cloning methods, it is now recognized that the majority of human cancer patients mount tumor-specific autoantibody responses (41). A wide variety of tumor Ags are recognized, including overexpressed proteins (e.g., HER-2/neu), aberrantly expressed proteins (e.g., cancer testis Ags), and a plethora of apparently normal self-proteins (41, 42). Furthermore, standard treatments for cancer, such as hormone and radiation therapy, can trigger additional autoantibody responses, presumably through presentation of dying tumor cells to the immune system (43).

Tumor-infiltrating B cells (TIL-Bs) are another important aspect of the B cell response to cancer. TIL-Bs have been studied most extensively in breast cancer, where they are present in \(\sim 25\%\) of tumors and comprise up to 40% of the tumor-infiltrating lymphocyte (TIL) population (42, 44, 45). TIL-Bs are often found in TLSs together with CD4+ and CD8+ T cells and DCs (46–48). TIL-Bs appear early during breast tumorigenesis, being present at the ductal carcinoma in situ stage (49). TIL-Bs generally express IgG and show evidence of Ag-driven expansion and somatic mutation consistent with affinity maturation (50–55). In one IgG sequencing study, \(> 45\%\) of TIL-B belonged to a clonal group, and there were 4–11 major clonal groups per tumor (46). In node-negative breast cancer, a gene signature indicative of TIL-B was positively associated with survival (56). Likewise, together with CD8+ and CD4+ T cells, TIL-Bs have been implicated in favorable survival rates in medullary breast cancer (42, 57, 58).

CD20+ TIL-Bs are also found in \(> 40\%\) of high-grade serous ovarian cancers, where they are associated with CD4+
and CD8\(^+\) T cells, as well as functional T cell markers such as TIA-1, granzyme B, and FoxP3 (59). TIL-Bs are strongly correlated with survival in ovarian cancer (59). Notably, tumors containing both CD8\(^+\) and CD20\(^+\) TILs are associated with higher survival than tumors containing CD8\(^+\) or CD20\(^+\) TILs alone, suggesting cooperative interactions between CD8\(^+\) and CD20\(^+\) TILs.

In non-small cell lung cancer, CD20\(^+\), CD8\(^+\), and CD4\(^+\) TILs are associated with increased survival (60, 61), as are TLS containing B cells, T cells, and mature DCs (62). TLS containing B cells, CD4\(^+\) and CD8\(^+\) T cells, and DCs are also found in colorectal cancer (63, 64). In cervical cancer, CD20\(^+\), CD4\(^+\), and CD8\(^+\) TILs are associated with a lower relapse rate (65). Finally, TIL-B are prominent in germ cell tumors, where they show evidence of Ag-driven clonal expansion and affinity maturation (66).

Considerable effort has gone into characterizing the target Ags of TIL-B. In a large study of various human cancers, TIL-B–derived autoantibodies were shown to react primarily with autologous tumor targets or allogeneic tumors of the same tissue type, suggesting they recognized tumor-associated Ags (67). In medullary breast cancer, TIL-B–derived autoantibodies were shown to recognize ganglioside D3 and \( \beta \)-actin, the latter by virtue of expression of \( \beta \)-actin on the surface of apoptotic tumor cells (54, 55, 68). In lung cancer, target Ags of TIL-B include p53, as well as many self-Ags that are overexpressed in tumor tissue (69).

In summary, TIL-Bs are prevalent in human cancer, recognize a wide variety of tumor and self-Ags, associate closely with T cells and other immune cells, and correlate with favorable outcomes. This provides clear rationale to better understand their mechanistic properties.

**Mechanisms of action of TIL-B**

Although little is known about the mechanisms by which TIL-B promote favorable outcomes in cancer, several possibilities are suggested by studies in autoimmunity, transplantation, and various experimental models (Fig. 1).

**TIL-B–derived autoantibodies**

As mentioned, autoantibodies and alloreactive Abs play major pathogenic roles in autoimmunity and transplantation, respectively. Similarly, TIL-Bs could potentially mediate their effects through autoantibodies, which could directly modulate the function of target proteins, or promote tumor immunity through the opsonization of tumor Ags, complement-mediated destruction of tumor cells, or Ab-dependent cytotoxicity. For example, in a murine study, adoptively transferred B cells promoted tumor rejection by producing complement-fixing, tumor-reactive Abs (70). Furthermore, the induction of autoantibody responses in mice through vaccination was shown to enhance CD8\(^+\) T cell responses against tumors (71). However, in human cancer, the relationship between autoantibodies and clinical outcomes remains controversial. For instance, anti-p53 serum autoantibodies, arguably the best-studied example, have been linked to favorable outcomes in some studies, but not others (72). This inconsistency may be attributable to the generally low concentrations of tumor-reactive autoantibodies in serum (42). Perhaps TIL-Bs, by virtue of location, raise the local concentration of autoantibodies to physiologically significant levels at the tumor site. Consistent with this idea, when human lung cancer specimens were engrafted in scid mice, TIL-B–derived autoantibodies were associated with decreased tumor growth (73, 74).

**Direct cytotoxicity by B cells**

TIL-Bs might also directly kill tumor cells through Ab-independent mechanisms (75). When stimulated with IL-21, human B cells can secrete granzyme B (76), which could potentially have direct cytotoxicity against tumor cells. Moreover, human B cells stimulated with IFN-\( \alpha \) or TLR agonist were shown to kill tumor cells directly via TRAIL signaling (77). Notably, however, cytokines derived from TIL-B can sometimes provide survival signals to tumor cells, as shown for lymphotoxin in murine prostate cancer (78).

**Immunoregulation by B cells**

Tumors typically contain complex mixtures of immune cells, which, in addition to TIL-Bs, can include cytotoxic T cells, Th cells (e.g., Th1, Th2, Th17), Tregs, DCs, and myeloid-derived suppressor cells. Thus, TIL-Bs could mediate their effects in part by regulating other immune cells. For example, under the influence of CD4\(^+\) Th1 and Th2 cells, B cells can be polarized into subsets that produce IFN-\( \gamma \), IL-12, and TNF-\( \alpha \) (Be-1 cells) or IL-2, IL-4, TNF-\( \alpha \), and IL-6 (Be-2 cells) (79, 80). Conversely, Be-1 and Be-2 cells can promote the differentiation of Th1 and Th2 cells, respectively, such that polarized cytokine profiles of both B cells and Th cells are amplified and maintained (79, 80). B cells also facilitate the formation of CD4\(^+\) T cell memory (81) and promote the survival and proliferation of activated CD8\(^+\) T cells through CD27–CD70 interactions (82). B cells also engage in negative regulatory relationships. For example, regulatory

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**FIGURE 1.** Potential mechanisms by which TIL-B promote tumor immunity. **Left panel,** TIL-B in human ovarian cancer visualized by anti-CD20 immunohistochemistry (brown). Original magnification \( \times 40 \). **Center panel,** Effector molecules produced by B cells. **Right panel,** Potential TIL-B mechanisms involving either direct effects on tumor cells or enhancement of the antitumor activity of T cells and other immune cells.
B cells can dampen immune responses through secretion of IL-10 and TGFβ-1 (7). Conversely, Tregs can inhibit B cell activation, proliferation, and Ab production (83).

In a striking example of immunoregulation, B cells can promote the formation of TLSs by secreting lymphotakin and chemokines, which attract and stimulate T cells, DCs, and other immune cells (7, 82, 84, 85). Accordingly, elimination of B cells in RA or renal allografts results in the disintegration of TLSs with consequent diminution of T cell activity (13, 16, 29, 30). Because TLSs are associated with favorable outcomes in human cancer (62) and murine models (86), this likely represents a key immunoregulatory property of TIL-B.

**Ag presentation by B cells**

Activated B cells can serve as APCs for both CD4+ and CD8+ T cells (7, 25). Indeed, B cells have an advantage over DCs, as they can selectively present cognate Ag collected through surface Ig molecules, which allows presentation of even low concentrations of Ag. Moreover, B cells can indirectly enhance Ag presentation by other APCs through production of specific Ab (87). The relative contributions of DCs and B cells to Ag presentation in vivo depends on context. In general, it appears DCs are important for initial T cell priming, whereas B cells may promote T cell expansion and memory formation (7, 25, 88).

Why might B cells be required as APCs at the tumor site? T cell activation and expansion are initiated by DCs in draining lymph nodes, yet there is growing recognition from viral models that optimal responses require a second wave of T cell proliferation triggered by APCs at the site of infection (89, 90). This process may be particularly important during prolonged or chronic immune responses (89). Whereas DCs might serve as effective APCs initially, one can imagine that with time, DCs may decline in number or become diverted to a suppressive phenotype, especially in the tumor environment. Although speculative, perhaps TIL-Bs can serve as local APCs under these circumstances, thereby sustaining the survival and proliferation of tumor-infiltrating T cells over the long term.

Consistent with this, we found that advanced ovarian cancers lack intratumoral DCs and instead contain TIL-Bs in close association with T cells (59).

**Conclusions**

The autoimmunity and transplantation fields have exposed B cells as key players in chronic, recalcitrant T cell responses. In addition to direct effects against tissues through Abs or cytotoxic pathways, B cells can promote T cell responses by producing cytokines and chemokines, facilitating TLS formation, and serving as APCs (Fig. 1). The contribution of B cells to tumor immunity might not be evident in many murine tumor models, which tend to involve rapid cytolytic responses akin to acute viral infections. By contrast, in human cancer, the beneficial effects of TLSs on clinical outcomes extend over many years, which is more consistent with a chronic immune process. From this perspective, how can we best engage B cells for cancer therapy? The Ab-mediated effects of TIL-Bs can potentially be mimicked by therapeutic mAbs, which represent a rapidly expanding class of cancer drugs. Furthermore, cell-based cancer vaccines can be used to elicit broader Ab responses that closely resemble those associated with naturally occurring TIL-Bs. For example, in melanoma and prostate cancer patients receiving cell-based vaccines expressing GM-CSF, the development of autoantibodies to self-antigens was associated with favorable clinical responses (91–93). It is less clear how best to enhance the immunoregulatory and Ag presentation functions of TIL-Bs. Models are needed in which TIL-Bs and T cells collaborate to provide long-term tumor control, as opposed to the models of acute rejection used by most investigators. To this end, TIL populations have been shown to persist for weeks to months in the most current tumor xenograft models (94), providing an experimental system to study functional interactions between TIL-Bs and other cell types. Additional insights will undoubtedly continue to emerge from the autoimmunity and transplantation fields through their continued efforts to disrupt the complex and powerful interactions between B cells and T cells.

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**Disclosures**

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