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Tumor-infiltrating B cells and T cells
Working together to promote patient survival

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We recently reported a novel cooperative relationship between tumor-infiltrating B cells and CD8+ T cells in ovarian cancer, leading to increased patient survival. Here, we discuss the mechanisms whereby B cells might enhance cellular immunity, including serving as antigen-presenting cells, organizing tertiary lymphoid structures and secreting polarizing cytokines. The enhancement of both B and T-cell responses may result in more potent and sustained antitumor immunity.

Numerous recent studies describe the strong association between tumor-infiltrating lymphocytes (TILs) and patient survival in human cancer.[1] Although most studies focus on CD8+ T cells, other lymphocyte subsets also contribute to this effect. Here, we briefly describe the roles of CD20+ tumor-infiltrating B cells (CD20+ TILs), which are strongly associated with favorable outcomes in breast, lung, ovarian and cervical cancer.[2] It is easy to rationalize the prognostic effect of CD8+ TILs, given their direct cytolytic activity against tumor cells. But how might CD20+ TILs promote tumor immunity? We recently investigated this issue in high-grade serous ovarian cancer (HGSC),[3] a setting where we had previously shown that CD20+ TILs are strongly associated with survival.[4]

First, we demonstrated that CD20+ TILs display characteristics of antigen-experienced, oligoclonal B cells. This is in contrast with the polyclonal mixture of naïve and memory cells that would be expected if B cells simply were irrelevant bystanders in the tumor environment. Specifically, we showed that CD20+ TILs express cell surface IgG, indicating that they have undergone class switching. Moreover, we sequenced the CDR3 regions of immunoglobulin-coding mRNAs, which revealed that CD20+ TILs are oligoclonal and have undergone somatic hypermutation.[3] Similar results have been reported in breast and germ cell tumors.[2] Thus, CD20+ TILs have hallmarks of antigen-experienced, clonally expanded B cells.

Drawing from the transplantation and autoimmunity fields, we considered several mechanisms to explain how CD20+ TILs could increase patient survival. Initially, we asked whether CD20+ TILs might be a source of tumor-specific serum autoantibodies, which are commonly found in patients.[5] Curiously, we found no association between CD20+ TILs and serum autoantibodies against two common tumor-associated antigens, NY-ESO-1 and p53.[3] Therefore, it appears that CD20+ TILs are not the main source of tumor-specific serum autoantibodies.

As CD20+ TILs are not responsible for humoral antitumor immunity, we reasoned that they may play a role in cellular immunity. In autoimmunity and transplantation, infiltrating B cells have been associated with tissue destruction and appear to enhance T-cell responses in part by serving as antigen-presenting cells (APCs).[6,7] Consistent with this, we found that CD20+ TILs express molecules associated with APCs, including MHC Class I and II, CD80, CD86 and CD40.[3] Moreover, by multicolor immunohistochemistry, we often found CD20+ TILs to localize with CD8+ T cells in loose aggregates within and adjacent to tumor islets.[3] Similar "tertiary lymphoid structures" have been reported in autoimmunity, allograft rejection and chronic infection.[8] Thus, CD8+ and CD20+ TILs co-localize in a pattern that is consistent with APC function.

These observations suggest that CD8+ and CD20+ TILs might work together to promote antitumor immunity and patient survival. We assessed this question by comparing survival in patients whose tumors contained CD8+ TILs with or without CD20+ TILs. Importantly, the presence of both CD8+ and CD20+ TILs was associated with markedly increased survival compared with CD8+ TILs alone or no TILs.[3] Collectively, our study provides strong evidence, albeit indirect, that CD8+ and CD20+ TILs act cooperatively to promote antitumor immunity.

How might CD20+ TILs help promote superior antitumor immunity? We propose three possibilities (**Fig. 1**). First, by serving as APCs, CD20+ TILs might facilitate the persistence of CD8+ T cells for long periods.[2] Whereas dendritic cells (DCs) may be well suited to initiate immune responses, protective antitumor immunity requires responses to persist for years. Perhaps CD20+ TILs provide ongoing stimulatory signals that inhibit the development of T-cell anergy or exhaustion. Moreover, B cells have the unique ability to take up specific antigen through

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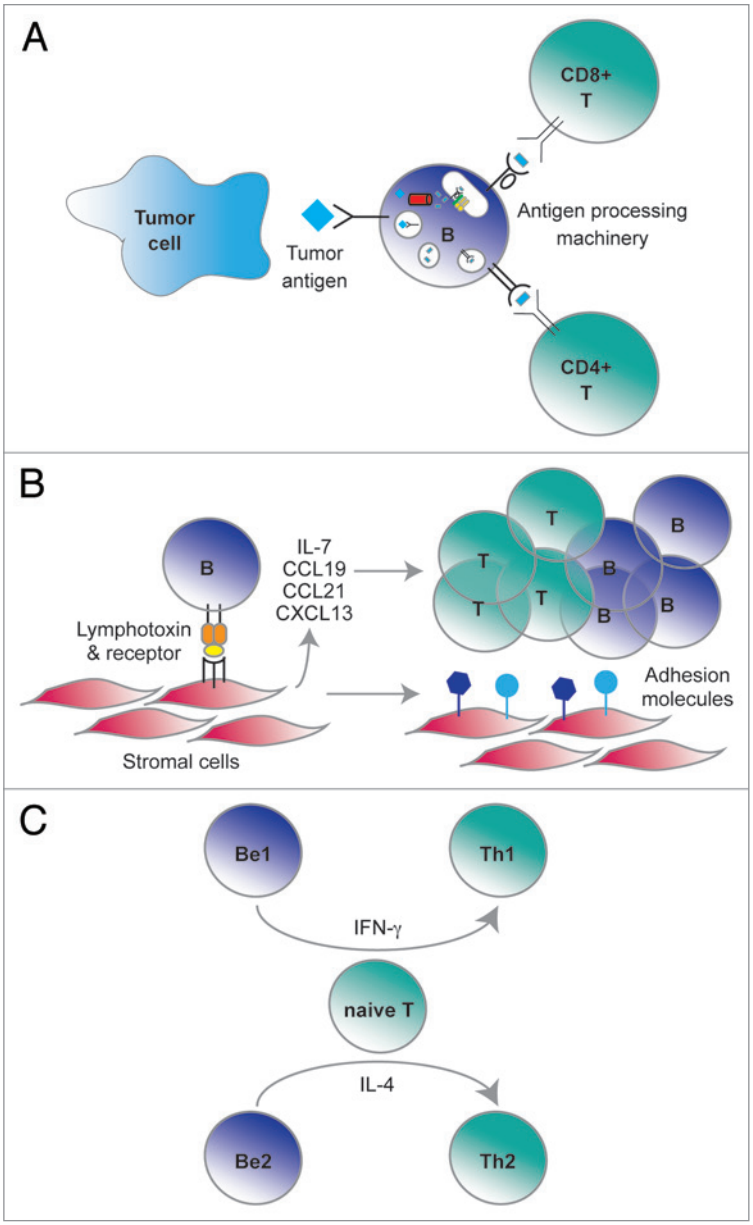

Figure 1. Three proposed roles for CD20⁺ tumor-infiltrating lymphocytes (TILs) in promoting anti-tumor immunity. (**A**) CD20⁺ TILs as antigen presenting cells. B cells can bind tumor antigens via surface Ig molecules, process them and then present peptides to CD8⁺ and CD4⁺ T cells via MHC Class I and Class II, respectively. (**B**) CD20⁺ TILs as lymphoid organizers. B cells are able to secrete lymphotoxin, which can induce stromal cells to express adhesion molecules, cytokines and chemokines. These factors, in turn, can recruit and retain other lymphocytes. (**C**) CD20⁺ TILs as polarizing cells. Type-I and Type-II B effector cells (Be1 and Be2) can secrete cytokines such as interferon γ (IFNγ) and interleukin-4 (IL-4), which can skew T-cell responses toward Th1, Th2 or other functional states.

their surface Ig molecules. This may allow concentration of low-level tumor antigens for processing and presentation to T cells. Second, B cells can produce cytokines such as lymphotoxin that promote the organization of local lymphoid structures.[6] Indeed, in autoimmunity, B cell depletion with rituximab disrupts T-cell infiltrates in affected tissues.[9] Third, B cells can produce cytokines that polarize T cells toward Th1, Th2 and maybe other functional phenotypes.[10] In summary, several unique properties of B cells might make them ideally suited to promote potent T-cell responses over the time frames associated with human cancer.

Looking ahead, what are the key research questions concerning CD20⁺ TILs? To definitively demonstrate that CD20⁺ TILs serve as APCs to T cells, it is imperative to identify their cognate antigens. Although two antigens have been identified in breast cancer, the antigen repertoire of CD20⁺ TILs remains largely undefined.[2] New high-throughput screening methods may now make antigen discovery more feasible. There is also an urgent need for understanding the functional profiles of CD20⁺ TILs. In HGSC, these cells show many of the hallmarks of memory B cells. However, they lack the canonical memory marker CD27,[3] a B-cell phenotype that is also observed in other pathological conditions such as systemic lupus erythematosus. This suggests that CD20⁺ TILs might have unique functional properties that warrant further study. Of key importance will be to define factors that facilitate the development of coordinated CD8⁺ and CD20⁺ TIL responses. With an improved understanding of these issues, it should be possible to design immunotherapies that enhance not only CD8⁺ T cell immunity against cancer, but also the potent contributions of CD20⁺ TILs.

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