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The many flavors of tumor-associated B cells

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Little is known on the role of distinct B-cell subtypes in human malignancies. We have recently performed a multiplex characterization of B cells in patient-derived tumor-associated tissues, documenting the activation and antigen-driven differentiation of B cells in metastatic lymph nodes and neoplastic lesions. Here we discuss the role of B lymphocytes as antigen-presenting cells and catalysts of T cell-based immunotherapies in view of these findings.

As opposed to that of the cellular immune system (in particular of T lymphocytes), the role of humoral immunity in tumor immunosurveillance has been poorly unexplored. Only recently, indeed, the prognostic impact of tumor-infiltrating B (TIL-B) cells has been recognized and their functional role has been begun to be investigated and discussed.[1,2]

Our recent work adds to the existing knowledge in two important ways. First, the role of B cells in metastatic tumors would be expected to extend beyond the primary neoplastic lesion. As in the case of T cells, the activation of B cells occurs in tumor-draining lymph nodes. In this setting, B cells can act as antigen-presenting cell (APCs) for T cells and, in return, receive signals that are required for affinity maturation, immunoglobulin class-switch and differentiation toward memory and plasma cells. Thus, the investigation of tumor-draining lymph nodes is pivotal for understanding the role of B cells in cancer.

Second, B cells come in different flavors, and TIL-B cells are likely to constitute an heterogeneous population, although they are often treated as a perfectly uniform cell type. Categorizing B-cell subpopulations has contributed to the understanding of conditions in which the humoral arm of the immune system plays a central pathophysiological role, such as common variable immunodeficiency[3] and graft- vs. -host disease.[4] With this in mind, we performed a multiplex characterization of B-cell subtypes in the neoplastic lesions, non-malignant tissues, lymph nodes and peripheral blood of patients affected by various solid tumors.[5] To this end, we emulated flow cytometry using the well-described Freiburg panel, which was originally introduced for the clinical diagnosis of immunodeficiency.[3] This classification reflects the antigen-driven differentiation of mature B cells and divides the $CD19^+$ B-cell population into the following subtypes; naïve B cells, class-switched memory cells, and plasmablasts. In addition, marginal zone-like B cells, rare $CD21^{low}$ B cells and immature, transitional B cells are also distinguished.

The 20 patients involved in this study (including 9 bladder carcinoma, 5 colon carcinoma, 4 melanoma, 1 pancreatic carcinoma and 1 prostate cancer patients) did not exhibit lymphopenia and they had normal $CD19^+$ B-cell counts in the peripheral blood. We compared the peripheral B-cell profiles of these subjects with that of healthy individuals, but did not observe any significant differences, with the exception of a lower proportion of plasmablasts and an increased spreading of the Igλ/Igκ light chain ratio among patient-derived samples.

When comparing metastatic and non-metastatic lymph nodes, the most striking observation was an increased proportion of plasmablasts in the latter. Furthermore, the $CD19^+$ cell population displayed an activated phenotype, as evidenced by an increased proportion of CD86-expressing cells.

Likewise, TIL-B cells showed signs of antigen-driven differentiation. In particular, the Freiburg classification displayed a distinctly right-shifted distribution, with increased proportions of switched memory cells and plasmablasts in neoplastic lesions than in non-malignant tissues of the same histology. Based on these observations, we proceeded by spectratyping and cloning the IgH chain in selected samples, and we were able to demonstrate a common clonal expansion in the neoplastic tissue of a bladder carcinoma patient as well as in the corresponding tumor-draining lymph node. The sequencing of the CDR3 region revealed a single base mutation in a WRCY hotspot, which is indicative of somatic hypermutation. This finding confirms previous results by other investigators, who also have detected clonal expansions among TIL-B cells and have demonstrated the reactivity against tumors of the corresponding immunoglobulins.[2] In addition, our data point to tumor-draining lymph nodes as the actual origin of TIL-B-cell clones.

In summary, we have demonstrated the emergence of antigen-driven B-cell

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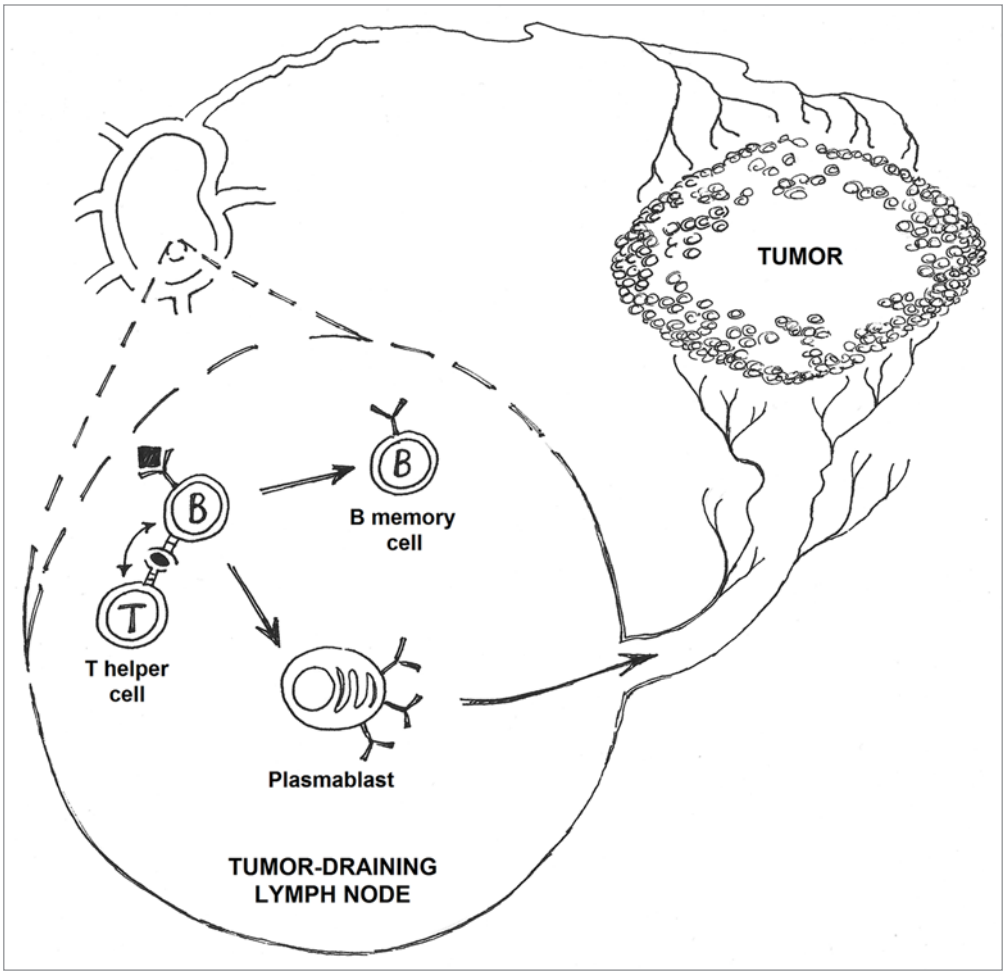

Figure 1. Activation of B cells in tumor-draining lymph nodes. Tumor cell debris is transported by afferent lymphatic vessels to draining lymph nodes, where tumor-associated antigens can be recognized by B lymphocytes via the immunoglobulins exposed on their surface. Antigen-bound immunoglobulins are then internalized and antigens are processed and presented on MHC class II molecules to $CD4^+$ T helper cells, resulting in the mutual activation of T and B lymphocytes. This promotes the differentiation of naïve B cells into memory cells and plasmablasts, which exit the lymph node and infiltrate neoplastic lesions to execute effector functions (e.g., antibody and cytokine secretion), as well as to present antigens to tumor-infiltrating T cells.

activation in tumor-associated tissues, suggestive of a T-cell dependent response (**Fig. 1**). Furthermore, our study substantiates the value of B-cell subtyping in dissecting the humoral immune response to tumors. Certainly, much remains to be explored in the B-cell compartment, including functional aspects such as cytokine production and regulatory features. Ample evidence from the field of autoimmunity supports the notion that targeting B cells can have profound effects on primarily T cell-driven pathogenic processes.[6] Obviously, the crosstalk between T and B cells is pivotal in this setting and our observation of a high proportion of CD86-expressing cells in tumor-associated tissues highlights the antigen-presenting qualities of B lymphocytes.

The relevance of antigen presentation by B cells has been recognized long ago,[7] but has somewhat fallen into oblivion, or at least, been overshadowed by the intense interest in dendritic cells. B cells bind cognate antigens by means of their cell-surface immunoglobulins. This high-avidity interaction sensitizes B cells to small amounts of antigens, which may be advantageous in the case of moderately immunogenic malignancies. Finally, B cells internalize antigen-bound immunoglobulins and process antigens to peptide fragments that are presented to antigen-specific $CD4^+$ T cells in the context of MHC class II molecules.

We have previously described the reactivity of unseparated sentinel lymph node cells from patients with colon and bladder carcinoma against malignant cells, constituting the basis for the subsequent development of a $CD4^+$ T cell-based adoptive immunotherapy.[8,9] During these ex vivo T-cell cultures the proportion of lymph node-derived B cells relative to other antigen-presenting cell subsets is high. Thus, our recent observations, possibly combined with novel techniques for B-cell cloning, may lead to the improvement of current adoptive anticancer immunotherapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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