OncoImmunology 2:10, e26066; October 2013; © 2013 Landes Bioscience

Tumor-infiltrating follicular helper T cells
The new kids on the block

Chunyan Gu-Trantien and Karen Willard-Gallo*

Molecular Immunology Unit; Institut Jules Bordet; Université Libre de Bruxelles; Brussels, Belgium

Keywords: anticancer immunity, breast cancer, CXCL13, follicular helper T cells, tertiary lymphoid structures, tumor-infiltrating lymphocytes

Abbreviations: GC, germinal center; SLO, secondary lymphoid organ; T_{FH}, follicular helper T; TIL, tumor-infiltrating lymphocyte; TLS, tertiary lymphoid structures

By analyzing $CD4^+$ lymphocytes in human breast carcinomas, we have recently uncovered the presence of follicular helper T cells in lesions exhibiting an extensive immune infiltrate. The presence of these specialized $CD4^+$ T cells, which localize to the germinal centers of peritumoral tertiary lymphoid structures found in extensively infiltrated neoplastic lesions, predicts improved disease outcome among breast carcinoma patients.

Solid neoplasms can elicit both anti- and pro-tumor immune responses, and such conflicting activities can often be detected within individual lesions.[1,2] Most past studies dealing with antitumor immunity have focused on $CD8^+$ cytotoxic T cells, as their cytotoxic activity was deemed critical for tumor eradication. Recent work indicates that specific subsets of $CD4^+$ T cells (namely, T_H1 cells), B cells, macrophages as well as dendritic cells provide an important contribution to antitumor immune responses as they secrete immunostimulatory factors or mediate antigen-presenting functions. Conversely, immunosuppressive cells including regulatory T cells, myeloid-derived suppressor cells and M2 macrophages have been ascribed with an important role for oncogenesis and tumor progression. While the balance between anti- and pro-tumor immune responses may dictate the elimination of malignant T cells during the early stages of tumorigenesis, the quantity of infiltrating lymphocytes (TILs) at surgery has been shown to predict disease outcome in patients affected by various solid neoplasms.[3] Thus, while antitumor immune responses generally fail to control the growth of primary tumors, cancer patients developing such responses to their neoplastic lesions have a better prognosis than patients that fail to do so. Indeed, at least theoretically, TILs might generate memory cells that mediate anticancer immunosurveillance upon tumor resection.

The key factors for the development and propagation of tumor-specific immunological memory remain unknown, although some clues are now emerging from studies of human malignancies. The peritumoral infiltrate of human lesions principally contains $CD4^+$ and $CD8^+$ T cells, sometimes in association with B cells. By studying human breast carcinoma (BC), we have recently discovered an important association between the presence of tumor-infiltrating $CD4^+$ follicular helper T (T_{FH}) cells, which localize to peritumoral tertiary lymphoid structures (TLS), and patient survival.[4] Our study aimed at producing a representative portrait of $CD4^+$ TILs in their native state by means of sensitive gene expression arrays, quantitative RT-PCR and flow cytometry, while minimizing ex vivo manipulation steps. In particular, we compared BC lesions exhibiting extensive vs. minimal lymphocytic infiltrates, finding that T_{FH} cells—which secrete the B cell chemoattractant chemokine (C-X-C motif) ligand 13 (CXCL13) specifically—correlate with extensive infiltration and the presence of TLS. Moreover, we demonstrated that tumor-infiltrating T_{FH} cells are associated with an increase in interferon γ (IFNγ)-producing T_H1 cells, $CD8^+$ T cells and B cells within neoplastic lesions, as well as with improved disease outcomes.

Recently discovered as an additional $CD4^+$ T cell subset, T_{FH} cells provide specialized help to B cells and are essential for the generation of memory B cells as well as long-lived antibody-secreting plasma cells. T_{FH} cells were initially identified in humans as chemokine (C-X-C motif) receptor 5 $(CXCR5)^+$ cells that home to B cell follicles in secondary lymphoid organs (SLOs). Since then, these specialized helper T cells have been extensively characterized in murine models, revealing that they are both required and limiting for the formation of germinal centers (GCs).[5] Together with follicular dendritic cells, T_{FH} cells also regulate the selection of somatically mutated antigen-specific

*Corresponding Author: Karen Willard-Gallo; Email: kwillard@ulb.ac.be; karen.willard-gallo@bordet.be
Submitted: 08/01/2013; Accepted: 08/05/2013
Citation: Gu-Trantien C, Willard-Gallo K. Tumor infiltrating T follicular helper cells: The new kids on the block. OncoImmunology 2013; 2:e26066; http://dx.doi.org/10.4161/onci.26066

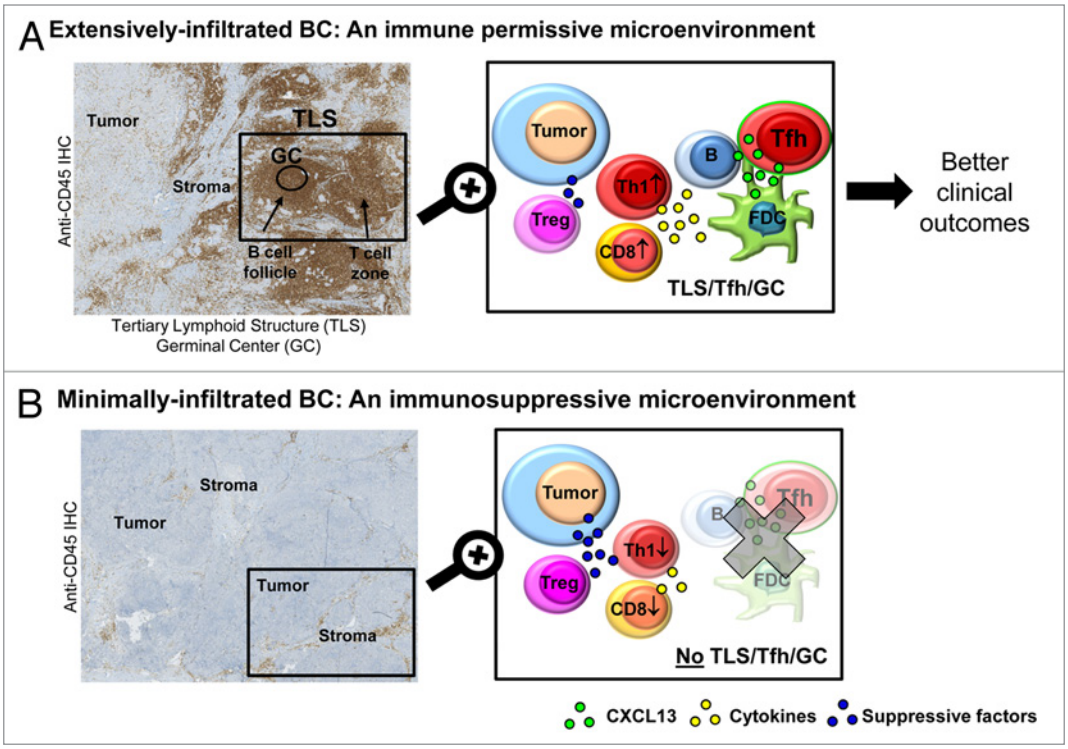

Figure 1. Immune infiltration of human breast carcinoma. (**A and B**) As opposed to minimally infiltrated human breast carcinoma (BC) lesions (**B**), extensively infiltrated BCs (**A**) contain peritumoral tertiary lymphoid structures (TLS) that comprise a T cell zone and a B cell follicle with active germinal centers (GCs). Extensively infiltrated BCs differ from their minimally infiltrated counterparts as they are characterized by a weakly immunosuppressive tumor microenvironment, elevated levels of activated effector T cells (including $CD4^+$ T_H1 as well as $CD8^+$ lymphocytes) that secrete antineoplastic cytokines such as interferon γ (IFNγ), as well as follicular helper T (T_{FH}) cells, residing together with B cells and follicular dendritic cells (FDCs) in GCs. In this setting, chemokine (C-X-C motif) ligand 13 (CXCL13)-producing T_{FH} cells appear to recruit B cells and guide their maturation into memory cells or antibody-producing plasma cells. The presence of peritumoral TLS with active GCs that contain CXCL13-producing T_{FH} cells is predictive of improved disease outcome among BC patients.

B cells that ultimately differentiate into high-affinity memory B cells or long-lived antibody-producing plasma cells. Notably, T_{FH} cells are key regulators of the protective B cell immunity induced by most human vaccines. Moreover, T_{FH} cells have also been linked with the development of autoimmune diseases (featuring the accumulation of T_{FH} cells) and immunodeficiency (accompanied by the loss of T_{FH} cells).[6] Our study on BC-infiltrating $CD4^+$ lymphocytes is the first to describe intratumoral T_{FH} cells in a non-hematological cancer and to show that their presence has a positive prognostic value (**Fig. 1**).

The significance of our observations might not be immediately apparent because humoral immunity has long been considered as the poor cousin in antitumor immunity.[7] Nonetheless, a recent study has clearly established a link between the presence of antigen-experienced tumor-infiltrating B cells and survival in ovarian cancer patients.[8] Our data suggest that the cooperation between T_{FH}-dependent B cell responses and T cell responses may be required for effective and sustainable antitumor immunity. The elicitation of adaptive immune responses in the structured environment of an SLO involves an orchestrated interplay between T cells, B cells and myeloid cells, resulting in the generation of effector and memory T cells in T cell zones as well as antibody-producing and memory B cells in GCs. We have recently demonstrated that $CD4^+$ T cells from extensively infiltrated BC lesions exhibit a more activated phenotype (less immunosuppressed) than those from minimally infiltrated tumors. Although TILs are generally viewed as functionally impaired or anergic, our study found that $CD4^+$ T cells in extensively-infiltrated BCs are principally housed in TLS and hence exhibit an improved activation profile. We provided experimental support for this concept by treating $CD4^+$ T cells from healthy individuals with supernatants from primary tumor cell cultures, largely recapitulating the profiles observed in vivo. Of note, these supernatants exhibited robust immunosuppressive effects on activated (as opposed to non-activated) $CD4^+$ T cells, with supernatants from minimally infiltrated tumors being the most efficient in this respect. Taken together, these observations suggest that lymphocytes housed in peritumoral TLS are activated in response to local antigens within a protected microenvironment. Once they leave the TLS and invade the immunosuppressive tumor bed, these cells may quickly undergo anergy but perhaps this is less relevant from a clinical perspective than their ability to retain immunological memory.

Ectopic or tertiary lymphoid structures that are structurally similar to SLOs not only have been identified at sites of autoimmune reactions in patients with rheumatoid arthritis or Sjögren's syndrome,[9]

but also have been associated with chronic inflammation in patients undergoing allograft rejection.[10] These TLS are thought to indicate a local chronic inflammatory response to persistent antigenic stimulation, and to mediate pathogenic effects through the aberrant secretion of immunoglobulins. TLS organization in BC lesions also mimic the architecture of a SLO, including a T cell zone and a B cell follicle that contains an active GC. The presence of T_{FH} cells/TLS in BC lesions (as detected by a genetic signature including 8 components) is indicative of good clinical outcomes, both for long-term survival in untreated BC patients or pathological complete responses in BC patients receiving neo-adjuvant chemotherapy. Although TLS may initially form adjacent to the tumor bed in the context of a chronic inflammatory response to malignancy (which generally favors disease progression), they may provide considerable benefits by sustaining the development of tumor-specific effector and memory lymphocytes.

Disclosure of Potential Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Scurr M, Gallimore A, Godkin A. T cell subsets and colorectal cancer: discerning the good from the bad. Cell Immunol 2012; 279:21-4; PMID:23041206; http://dx.doi.org/10.1016/j.cellimm.2012.08.004
2. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro-versus anti-tumor immunity. Cancer Metastasis Rev 2010; 29:309-16; PMID:20405169; http://dx.doi.org/10.1007/s10555-010-9223-6
3. Jochems C, Schlom J. Tumor-infiltrating immune cells and prognosis: the potential link between conventional cancer therapy and immunity. Exp Biol Med (Maywood) 2011; 236:567-79; PMID:21486861; http://dx.doi.org/10.1258/ebm.2011.011007
4. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, Ravoet M, Le Buanec H, Sibille C, Manfouo-Foutsop G, et al. CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest 2013; 123:2873-92; PMID:23778140; http://dx.doi.org/10.1172/JCI67428
5. Ramiscal RR, Vinuesa CG. T cell subsets in the germinal center. Immunol Rev 2013; 252:146-55; PMID:23405902; http://dx.doi.org/10.1111/imr.12031
6. Tangye SG, Ma CS, Brink R, Deenick EK. The good, the bad and the ugly - TFH cells in human health and disease. Nat Rev Immunol 2013; 13:412-26; PMID:23681096; http://dx.doi.org/10.1038/nri3447
7. Nelson BH. CD20+ B cells: the other tumor-infiltrating lymphocytes. J Immunol 2010; 185:4977-82; PMID:20962266; http://dx.doi.org/10.4049/jimmunol.1001323
8. Nielsen JS, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, Nelson BH. CD20+ tumor-infiltrating lymphocytes have an atypical CD27- memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. Clin Cancer Res 2012; 18:3281-92; PMID:22553348; http://dx.doi.org/10.1158/1078-0432.CCR-12-0234
9. Corsiero E, Bombardieri M, Manzo A, Bugatti S, Uguccioni M, Pitzalis C. Role of lymphoid chemokines in the development of functional ectopic lymphoid structures in rheumatic autoimmune diseases. Immunol Lett 2012; 145:62-7; PMID:22698185; http://dx.doi.org/10.1016/j.imlet.2012.04.013
10. Thaunat O. Pathophysiologic significance of B cell clusters in chronically rejected grafts. Transplantation 2011; 92:121-6; PMID:21555973; http://dx.doi.org/10.1097/TP.0b013e31821f74fe