

## Tumor-infiltrating B cells come into vogue

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Author contributions: Linnebacher M designed, drafted, revised and finally approved this paper.

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Received: October 16, 2012 Revised: November 8, 2012

Accepted: November 14, 2012

Published online: January 7, 2013

### Abstract

Lymphocyte infiltration into solid tumors has been recognized as a main determinant of positive prognosis. For the most part this is attributed to cytotoxic T cells capable of directly destroying malignant cells. However, when considering the complex composition of the human immune system, recent findings of Nielsen *et al* on a potentially central role of tumor-infiltrating B cells is not really surprising. In this commentary article, I want to highlight the enormous potential impact of this observation for basic and translational research, prognostic procedures and ultimately for the development of future therapeutic concepts.

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**Key words:** CD20<sup>+</sup> tumor-infiltrating lymphocytes; Tumor-infiltrating B cells; Prognosis; Immunotherapy; Functional immunology

Linnebacher M. Tumor-infiltrating B cells come into vogue. *World J Gastroenterol* 2013; 19(1): 8-11 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i1/8.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i1.8>

### INVITED COMMENTARY ON HOT ARTICLES

Most previous studies on the infiltration of lymphocytes into human malignant tumors focused on CD8<sup>+</sup> T cells, the cell type with best antitumoral potential. And for many tumors, the degrees as well as the composition of tumor-infiltrating lymphocytes (TIL) have strong positive prognostic relevance<sup>[1]</sup>. However, recent findings established tumor-infiltrating B cells as next-best predictor of a positive patient outcome<sup>[2-4]</sup>. In their very recent publication, Nielsen *et al*<sup>[5]</sup> analyzed CD20<sup>+</sup> lymphocytes infiltrating into ovarian cancer. It is one of the very few detailed and comprehensive analysis of tumor-infiltrating B cells and moreover, it raises the question of functionality. The authors end up not only with confirmation of a strong association of CD20<sup>+</sup> TILs' presence and prolonged survival but with several findings shedding light into the true function of B cells in the immune systems' fight against cancer.

#### Major findings

First, they confirm earlier work demonstrating antigen-maturation and an activated phenotype of CD20<sup>+</sup> TIL [immunoglobulin (Ig)D<sup>+</sup>, IgM<sup>+</sup>, IgG<sup>+</sup>]<sup>[6-8]</sup>. Sequencing of the immunoglobulin genes consequently reveals high levels of somatic hypermutation in TIL B cells. This has also frequently been described for mammary carcinoma<sup>[6,7,9-12]</sup>. Thus the authors conclude that CD20<sup>+</sup> TILs have undergone activation, Ig class switching, somatic hypermutation, and clonal expansion; all of which are hallmarks of antigen exposure.

Furthermore, detailed analysis of the B cells' phenotype shows that naïve B cells were absent and only marginal amounts of germinal center B cells and plasmablasts (CD38<sup>+</sup> but CD138<sup>-</sup>) were present. The vast majority of CD20<sup>+</sup> TILs were memory B cells (IgD<sup>+</sup>, CD38<sup>low</sup>) of an atypical type since they did not express the canonical memory marker CD27. The authors explain this finding

with either recent activation of B cells, since exposure of B cells to CD70<sup>+</sup> T cells leads to rapid CD27 down-regulation. Alternatively, the lack of CD27 expression on CD20<sup>+</sup> TIL may allow foregoing helper T cell-induced antibody production by ligation of CD27 with CD70. They conclude that CD20<sup>+</sup> TILs may thus directly contribute to cellular immunity. Other markers were expressed as expected from B cells: human leukocyte antigen (HLA)-ABC, HLA-DR, CD40 as well as moderate CD80 and CD86. This finding was interpreted as prerequisite for and hint towards functional antigen-presentation of the CD20<sup>+</sup> TIL to T cells in the tumor environment.

Finally, Nielsen *et al* observed an activated effector phenotype (HLA-DR<sup>+</sup>, CD45RO<sup>+</sup>, CD45RA<sup>-</sup>, CD62L<sup>-</sup>) of tumor-infiltrating CD4<sup>+</sup> and CD8<sup>+</sup> T cells. This finding is again consistent with the literature<sup>[13]</sup>. Moreover, they describe frequent co-localization of CD8<sup>+</sup> and CD20<sup>+</sup> lymphocytes both in the tumor stroma and epithelium. Clinical relevance comes from the fact that patients' survival is best when both CD8<sup>+</sup> and CD20<sup>+</sup> lymphocytes are present. The authors conclude that CD20<sup>+</sup> TIL most likely have a function as professional antigen presenting cells (APC) to support the T cells in the immune systems' fight against cancer. The latter is of extreme interest in the context of the development of novel immunotherapies. Even though dendritic cells (DCs) are established as the most potent and thus possibly also most important professional antigen presenting cell type, antigen-specific B cells may be similar if not more important in the body's fight against cancer cells. This assumption may seem a bit provocative, but Nielsen *et al*<sup>[5]</sup> hint towards the unique potential of B cells to concentrate the level of an antigen presented to T cells by membrane-IgG-guided antigen capture. Since the levels of tumor-released antigens are virtually highest in the tumor bed, it seems only logical for the CD20<sup>+</sup> antitumoral B cell to "work there".

#### **Impact on future basic and translational research**

The authors arrive at the conclusion that it will be necessary to identify the cognate antigens of CD20<sup>+</sup> TILs and to assess recognition by CD4<sup>+</sup> and CD8<sup>+</sup> TILs in order to definitively show that CD20<sup>+</sup> TILs serve as APC in the tumor environment. It will be a demanding task demonstrating simultaneous recognition of a given tumor antigen at the tumor site by all the cellular players involved: CD4<sup>+</sup>, CD8<sup>+</sup> and CD20<sup>+</sup> TILs. Technically, this can be solved either by recombinant molecular biological tools, i.e., presence of T and B cell receptors. However, this approach is complicated by the fact that the exact receptor rearrangements should be known in advance. Alternatively, functional cellular tests using CD20<sup>+</sup> TIL B cells as APC to stimulate CD4<sup>+</sup> as well as CD8<sup>+</sup> TILs may also lead to the identification of antigens relevant for the immunological control of a given tumor. In our recent study on tumor-infiltrating B cells of colorectal cancer<sup>[14]</sup>, we presented a simple method to immortal-

ize and clone tumor-infiltrating B cells. The potential of these clones to functionally influence tumor growth either directly or in mutual interaction with T lymphocytes will be an interesting field of research<sup>[15]</sup>. Beside antigen identification, *in vitro* analysis using cell-cell interaction assays and even functional *in vivo* tests will be possible when using matched tumor cell lines or established tissue lines of primary tumor pieces engrafted in immunodeficient animals<sup>[16]</sup>.

Another interesting aspect lies in the mere frequency of CD20<sup>+</sup> TILs in comparison to DCs; the latter are much less prominent in ovarian cancer. Many of the studies on DCs infiltrating tumor tissues used CD83 as a typical DC marker<sup>[17]</sup>. We frequently observed CD83-expression on the above mentioned *ex vivo* cloned and immortalized B cells<sup>[14]</sup>. Simultaneously, many of these clones totally or at least partially failed to express the classical B cell markers CD19 and CD20<sup>[14]</sup>. Although the marker expression may be influenced by the *in vitro* culture, one may still speculate on tumor-infiltrating B cells falsely counted as DCs - at least when CD83 was used as DC marker. Multi-color stainings on cryosections will help to clarify this question. Similarly, Nielsen *et al* shed light into another open question: which functional subtypes of B cells infiltrate tumor tissues? To definitely answer this, our above mentioned *ex vivo* cloning strategy will, together with classical analyses of different surface markers by immunohistochemical staining, possibly be very helpful.

#### **Impact on future diagnostic procedures and prognosis**

The contribution of B cells present in tumor tissues on the outcome of the disease is still largely cryptic. The data of Nielsen *et al*<sup>[5]</sup> clearly support a positive prognostic value of CD20<sup>+</sup> TIL in ovarian cancer. Together with recent similar findings of other groups on head and neck cancer<sup>[18]</sup>, cutaneous melanoma<sup>[19]</sup> and breast cancer<sup>[20]</sup>, it is likely that the presence of CD20<sup>+</sup> TIL may be a good prognosticator in general. Moreover, all the above mentioned analyses clearly focus on the B cell marker CD20 and broader analyses using B cell subset markers are definitely warranted in order to allow for diagnostic values of B cell subtypes infiltrating different cancers.

Contrary to these findings, it has been shown that B cells' presence in tumor draining lymph nodes is associated with lymphangiogenesis in a mouse model, which subsequently increases metastasis<sup>[21]</sup>. In human cancer, a metastasis-promoting role is discussed for B1 lymphocytes in melanoma<sup>[22]</sup> and B cell-secreted lymphotoxin can promote castration-resistant prostate cancer<sup>[23]</sup>. Taken together, these findings clearly imply that B lymphocytes' subtypes and most likely additional tissue context parameters will be predictive for either antitumoral or tumor-promoting activity of tumor-infiltrating B cells.

#### **Impact on future therapeutic concepts**

In my opinion, the most interesting argument brought forward by Nielsen *et al*<sup>[5]</sup> is the following: during pro-

longed immune responses as observed in autoimmunity, allograft rejection as well as chronic infection, B cells and T cells form so-called tertiary lymphoid structures. Thus, both cell types maintain a strong immune response in cooperative interactions at the affected site. The authors suggest that future antitumoral immunotherapies should be designed as to mimic these chronic immune reactions: T-cell responses are sustained over many months or years by direct interactions with co-localized B cells. Thus, it may be possible to generate more potent, sustained T-cell responses in the tumor environment by promoting the infiltration of tumor-reactive B cells. This line of argumentation is well taken, since in some colorectal cancers tumor-infiltrating B cells typically reside in tertiary lymphoid structures together with other tumor-infiltrating leukocyte populations thus permitting anti-tumor effects. This so-called “Crohn’s like” reaction has repeatedly been associated with very good prognosis for colorectal cancer<sup>[24]</sup> and is especially associated with the molecular subtype with best prognostic behaviour; i.e., microsatellite-instability<sup>[25]</sup>.

Another very promising strategy may base on the above mentioned unique potential of B cells to specifically concentrate antigens *via* their membrane-bound Ig molecules. I would like to speculate that tumor-antigen-specific B cells will have an enormous T cell stimulatory potential. Thus, cellular therapies can be envisioned basing on isolation and expansion of tumor-infiltrating and antigen-specific B cells.

In summary, improved understanding of the functional phenotype of tumor-infiltrating B cells (CD20<sup>+</sup> but possibly also CD20<sup>neg</sup> B cells), their target antigens, and their mechanism of recruitment to target tissues may not only help to understand the role of B cells in the natural course of tumor diseases but also will have potentially strong impact on patients’ diagnosis and last but not least will facilitate the design of more effective immunotherapies for the treatment of cancer.

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