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CD20+ T cells in Primary Mediastinal Large B Cell Lymphoma Microenvironment

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Dear Editor:

We have read the article entitled "Evaluation of primary mediastinal large B cell lymphoma by flow cytometry" recently published in Cytometry Part B with great interest (1). In this paper, Cherian & Fromm along with neoplastic clone evaluated the reactive infiltrate in primary mediastinal large B cell lymphoma (PMLBCL). The authors reported presence of CD3+CD4+CD7-bright/CD45-bright subpopulation in the reactive infiltrate in 41% of their cases, which appears to be a common finding in classical Hodgkin lymphoma tumor microenvironment.

We observed CD20+ T cells in the mediastinal mass tissue in two recent cases with histologically-proven PMLBCL on flow cytometric analysis. CD20+ T cells were identified by CD3, CD7 and CD2 staining (Figure 1). These two cases with prominent CD20+ T cell population (45.53% and 56.3% within the CD45-bright lymphocyte gate) showed decreased CD4/CD8 ratio (0.49 and 0.35, respectively) in contrast to the findings recently reported in PMLBCL reactive tissue (1). In case 1, the CD20 expression on T cells was about the same intensity as that of mature B cells and about one logarithmic difference lower than that of the neoplastic cells, while in case 2 the CD20

expression on T cells was broad compared to that of mature B cells within the reactive infiltrate (Figure 1A and 1B). The specimen in case 1 was run and analyzed on Beckman Coulter Epics XL flow cytometer (Brea, CA) on 3 color panel and on Beckman Coulter Gallios flow cytometer (Brea, CA) on 10 color panel in case 2. For both panels, FMO (fluorescence minus one) controls were run to account for any artifact or nonspecific fluorescence. Three different fluorochrome conjugates of CD20 monoclonal antibody, all with the same clone (Clone B9E9, Beckman Coulter, Brea, CA) were used in the panels – APC and APC Alexa Fluor 750 in Case 1 and PE in case 2.

Other B cell markers such as CD19 and CD22 were absent on these cells suggesting that these were CD20-expressing T cells and not B and T cell-cell or cell particle-cell conjugates (Figure 1C). 10-color flow cytometric analysis of CD20+ T cells showed that these were distributed among various T cell subsets, including CD4-CD8- (double-negative), CD4+CD8+ (double-positive), CD8+ and CD4+ cells, 42.86%, 5.08, 39.85% and 12.22%, respectively in case 2. (Figure 1D). Interestingly, in the second case, there was an increased double-negative T cell presence (25% of T cells) with positive TCR-□□staining pattern (90%) in the tumor microenvironment. To our knowledge these findings have not been reported in PMLBCL or any other type of lymphoma reactive tissue.

A small population (~3-5%) of CD3+CD20dim+ has been identified in peripheral blood of healthy individuals, cerebrospinal fluid and primary and secondary lymphoid tissue, including thymus (2, 3). It has been shown that, immunophenotypically these T cells are more common in CD8+, CD45RO+ memory, and in CCR7− subpopulations. Compared to CD3+CD20- T cells, they show increased frequency of IL-4, IL-17, IFN-γ, and TNF-α producing cells (3).

Origin of these cells is controversial. One study has suggested that these cells may arise from transfer of CD20 molecules to T cells upon close T cell/B cell interaction in the tissue by intercellular exchange of membrane components via a process known as trogocytosis (4). However, another study showed that peripheral blood CD3+CD20+ cells transcribe both CD3 and CD20 (3).

The function of CD20+ T cells is not yet clear. This subset has been found to be in a higher state of activation in patients with autoimmune disorders such as psoriasis, rheumatoid arthritis, multiple sclerosis and increased in ovarian cancer. Compared with healthy individuals, circulating CD20+ T cells have been shown to produce more cytokines, interleukin IL-17A, tumor necrosis factor (TNF)-α and IL-21 in patients with psoriasis. Additionally, higher levels of IL-17A, TNF-α and IL-21 production by CD20+ T cells was observed in the affected areas and skin lesions with increased severity index scores in psoriasis (5). In multiple sclerosis, activated CD20+ T cells have been shown in

chronic lesions of brain, secreting pro-inflammatory cytokines IFN-γ and IL-17, possibly contributing to the disease pathogenesis (6). Eggleton and colleagues have reported that the proportion of IL-17-producing CD20+ T cells in the peripheral blood of rheumatoid arthritis patients increased by 240-fold as compared to healthy individuals (7). In ovarian cancer, while CD20+ T cells were 6% in peripheral blood, this population was found to be increased to 23% in ascites fluid (4).

Several cases of CD20+ T cell leukemia/lymphoma have been reported in the literature (8- 13). Quintanilla-Martinez et al. speculated that CD20+ T cell lymphomas may arise from neoplastic transformation of normal CD20+ T cell subset (8). In PMLBCL, it is also possible that CD20+ T cells are highly activated Th17 cells and may be involved in anti-tumor immunity. Studies in larger number of cases analyzing the tumor tissue and the effect of CD20+ T cells in treatment outcome are warranted, which might help guide therapy including, the use of immunotherapy and immune checkpoint inhibitors, in particular (14).

Conflict of interest: Nothing to report.

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Figure Legend

Fig1. Two cases of PMLBCL evaluated by flow cytometry

A. Case 1 displaying CD20+ CD7+ T cells within the reactive infiltrate of the tumor tissue. The CD20+ T cells have about same intensity of CD20 expression as that of mature B cells within the reactive infiltrate, while one log difference lower CD20 intensity than that of neoplastic cells.
B. Case 2 displaying CD20+CD3+ T cells within the reactive infiltrate of the tumor tissue. The CD20+ T cells show broad CD20 expression. Tumor cells were not detected in the tumor tissue specimen on the flow cytometric analysis.
C. CD20+ T cells in Case 2 does not show any significant expression of other B cell markers such as CD19 and CD22.

D. CD20+ T cells in Case 2 showed that these cells were distributed among various subsets of T cells, including CD4+, CD8+, CD4+CD8+ (double positive) and CD4-CD8- (double negative) T cells.