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CD8 expression on clonal b-cll cells and CD8+ lymphocytes in the microenvironment, relations with prognosis and survival

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ABSTRACT

In lymphoproliferative neoplasms including chronic lymphocytic leukemia (CLL), hairy cell leukemia and even multiple myeloma, the biology of the monoclonal cells as well as the surrounding cells which are called microenvironment of the bone marrow are known to be in an interplay.

Data of 275 patients diagnosed with B-CLL were collected in a retrospective manner. Demographic features, laboratory including flow cytometry, immunoglobulins, genetic evaluations and clinical follow up were recorded from files.

111 patients were female (40.4%) while 164 were male (59.6%). Mean age was 70.46 years (21-92).

CD8 positivity on CLL cells was observed in 12 patients. No significance was observed regarding complications and survival. 71 patients demonstrated a dense surrounding CD8 positive lymphocytes. Of these patients, none had 17p deletion while 28 patients demonstrated 13q deletion (39.4% of CD8+ Treg group while 82.3% of all patients with 13q del) and 6 patients had 11q deletion. ITP, lowIgG levels, development of secondary malignancies, high percentage of bone marrow infiltration, Richter's transformation were significantly corelated. CD8+ T lymphocyte richness in the microenvironment was significantly related with survival.

Cancer is related with immunodefiency. With the evolution of tumors, they find ways to elude immune recognition. CD8 expression on B-CLL cells may not be explained with clinical features or prognosis. But the density of CD8 within the infiltrated bone marrow may explain the long term immune escape of CLL cells, with the effort to balance immune regulation by disease control, though resulting with increased autoimmunity.

Key Words: Chronic, Lymphocytic, Leukemia, B-Cell CD8+ T lymphocytes, Immune dysregulation

Introduction

In lymphoproliferative neoplasms including chronic lymphocytic leukemia (CLL), hairy cell leukemia and even multiple myeloma, the biology of the monoclonal cells as well as the surrounding cells which are called microenvironment of the bone marrow are known to be in an interplay. Before the understanding of the importance of microenvironment, these malignancies were treated with cytotoxic agents which are aimed towards the actively DNA replicating cells. As the low grade lymphoproliferative malignancies replicate much slower, these agents generally did not achieve an adequate response despite their heavy treatment related burdens like toxicities.

The need to treat slow replicating tumors led to other objectives, like targeted treatments for specific mutations and the modulation and even education of the microenvironment, making the immune system develop an anti-tumor response. This idea has led to genetic engineering and the development of chimeric antigen receptor (car) t-cell therapies which the future of hematology now depends.

In this perspective, we aimed to investigate the innate immune response to CLL as well as the additional properties these monoclonal B-CLL cells may have acquired during the course of the disease and their implications on prognosis, complications and survival.

Materials and Methods

Data of 275 patients diagnosed with B-CLL were collected in a retrospective manner. Demographic features, laboratory including flow cytometry, immunoglobulins, genetic evaluations and clinical follow up were recorded from files. Flow cytometric analysis was performed on bone marrow samples at the time of diagnosis with Becton Dickenson (BD FACSCalibur platform) analyzer. Four color flow cytometric analysis has been used with fluoroscein isothiocyanate (FITC), R-phycoeryhtrin (PE),

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Table 1. Descriptive analysis of patients

Gender (Female/Male)	111 (40.4%)/164 (59.6%)
Mean Age (years)	70.64 (21-92)
Genetic analysis	
17p deletion	7 patients
13q deletion	34 patients
11q deletion	21 patients
Richter's transformation	44 patients
ITP	72 patients
AHA	12 patients
CD8 positivity on clonal cells	12 patients
CD8 dense surrounding cells	71 patients

Table 2. Statistical Analysis of CD8-Dense Microenvironment and Relations with Clinical Features

	CD8 dense environment positive patients	CD8 nondense environment patients	Total
17p deletion	1	6	7
13q deletion	28	6	34
11q deletion	6	15	21
ITP	30 (p=0.000)	42	72
Low IgG levels	55 (p=0.000)	82	137
Bone marrow infiltration >60%	26 (p=0.000)	31	57
Richter's transformation	19 (p=0.004)	25	44
AHA	1 (p=0.308)	11	12
Secondary malignancy	14 (p=0.172)	26	40
Hemoglobin >13 g/dL	34 (p=0.311)	109	143
Lymphocyte count	(Pearson corelation) p=0,762		

allophycocyanin (APC) and peridinin chlorophyl protein (PerCP).

Flow cytometry: As gating strategy, universal approach for lymphocyte phenotyping is forward vs right angle light scatter. In our center, we prefer side scatter and CD45 APC combination for leucocyte separation. After gating lymphocytes with side scatter and CD45 APC, monoclonality is observed with CD19 FITC + CD5 PE. Gating for further immunophenotyping was performed with this monoclonality determination. CD8 expression in this monoclonal cells were determined with a cutoff as 20%, which is accepted by using an absolute positive isotype control and comparing our samples with this positive control.

After the determination of B-CLL with this universal technique, we retrospectively analyzed our readings with gating all the cells besides B-CLL cells in the side scatter and CD45 APC and determined CD8 positive T cells as percentage within the bone marrow samples (Figure 1).

Statistical analysis: IBM SPSS V20 were used for statistical analysis. Parametric variables were compared with chi-square test and nonparametric

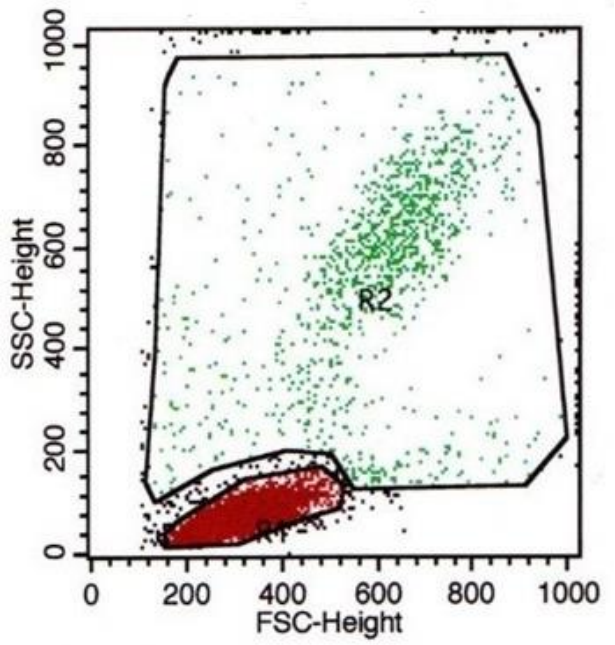

Fig. 1. Flow cytometric gating evaluation of clonal cells and surrounding cells

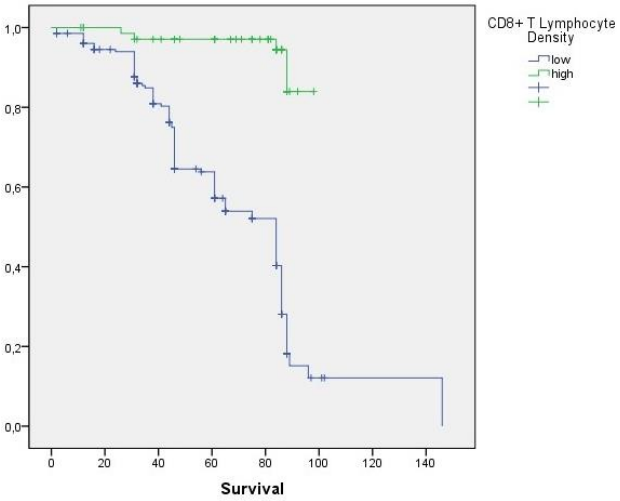

Fig. 2. Effect of CD8+ lymphocytes in the microenvironment on survival

variables were analyzed with Mann-Whitney U test. Logistic regression analysis was performed for significant variables and Kaplan Meier analysis was performed for survival.

Results

Data of 275 patients with CLL were evaluated in a retrospective manner. 111 patients were female (40.4%) while 164 were male (59.6%). Mean age was 70.46 years (21-92). 17p deletion was observed in 7 patients, 13q deletion in 34 and 11q deletion was observed in 21 patients. Richter's transformation was observed in 44 patients, immune thrombocytopenia

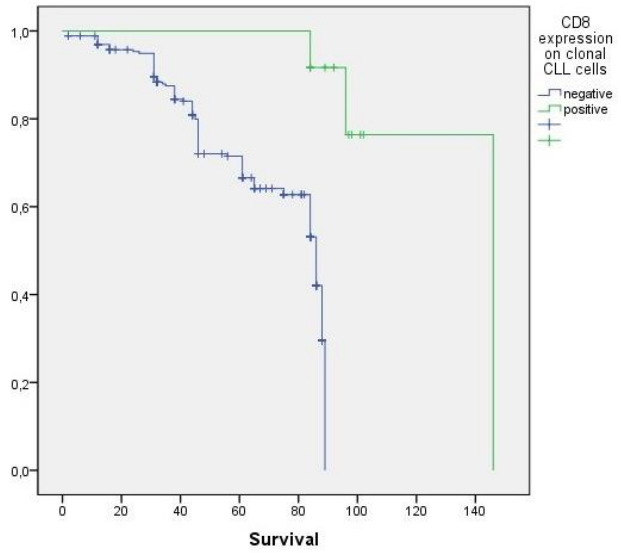

Fig. 3. Effect of CD8 expression of clonal CLL cells on survival

(ITP) in 72 patients and autoimmune hemolytic anemia (AHA) in 12 patients.

CD8 positivity was observed in 12 patients. Within these patients, 1 was 17p del positive, with Richter's transformation. Within these patients, only 2 presented with Rai stage 3 or 4 disease, hemoglobin level >10 g/dL and thrombocytes >100.000/mm3. 1 had ITP and none had AHA. These observations when compared to the CD8 negative group, did not show statistical significance.

After the evaluation of CD8 expression of monoclonal CLL cells, surrounding cells were gated by flow cytometry. 71 patients demonstrated a dense surrounding CD8 positive lymphocytes (Tregs). Of these patients, 1 had 17p deletion while 28 patients demonstrated 13q deletion (39.4% of CD8+ Treg group while 82.3% of all patients with 13q del) and 6 patients had 11q deletion (Table 1). ITP, lowIgG levels, high percentage of bone marrow infiltration, Richter's transformation were significantly corelated with CD8 positive microenvironment (p levels; 0.000; 0.000; 0.004; 0.000; 0.000 respectively) while AHA, development of secondary malignancies, hemoglobin and peripheral lymphocyte levels were not (Table 2). Kaplan Meier analysis showed that CD8 expression on monoclonal cells was not related with survival while CD8+ T lymphocyte richness in the microenvironment was significantly related with survival (Figure 2 and 3).

Discussion

Cancer is related with immunodefiency. With the evolution of tumors, they find ways to elude immune recognition (1). The complex scheme used by tumoral

cells to breakout from immune response include the production of proinflammatory cytokines, modulation of regulatory T cells (Tregs) and recruitment of tumor-associated macrophages (TAMs) (2,3). With the perspective of undoing the immune escape of tumors, reconstruction of immunomodulation has now found its place in the treatment of various hematological malignancies as wells as benign disorders like ITP.

B-cell CLL is characterized by the slow accumulation of lymphocytes with mature appearance. Their long term presence in bone marrow and circulation is an example of tumor cells escaping immune recognition. In regardless of disease progression, CLL is strongly associated with immune dysregulation, both as an immune suppression also as an immune over reaction (4,5). Abnormalities related with T-cell mediated immunity and adaptive responses are now accepted as common and typical features of CLL (6). Certain subsets of lymphocytes have been investigated in the immunosuppression of CLL. In 1970, Gershon et al have suggested the role of thymic lymphocytes in the cell interaction of immune tolerance (7) which is a historical paper leading to further studies on subsets of lymphocytes and their immune functions. Tregs are subset of T cells which are characterized immunophenotipically as CD4+ CD25+ FOXP1+ and CD127dim/- are thoroughly investigated in CLL and are found to be responsible in the immune dysregulation of CLL (8-13).

Tregs show mainly immunosuppressive roles in the system particularly in cancer biology and autoimmunity (8). Suppressing anti-tumor immune response, they suppression of other T cells, B cells and dendritic cells, increased number of Tregs are observed in patients with solid or hematological malignancies which suggest an essential role in cancerogenesis.

Besides Tregs, NK and CD8+ T cells are also been investigated. CD8 cells were observed to be related with prognosis (14-16). The development of immune cells in CLL may be associated with the clonal evolution of B cells. We also observed the importance of CD8+ T cells within our group as the density of the CD8+ cells increase, prognosis is much better but also with a cost of increase in autoimmunity. These cells within the environment of CLL cells may be immunologically educated and their effects of immune recognition and killing of tumor cells may be enhances by immunomodulation (3).

Expressing a T cell related surface antigen may be accepted as aberrant. Mature looking CLL lymphocytes may be hit clonally in a stage where they have been introduced with differentiation molecules. Expression of a dendritic surface molecule, CD11c on CLL has been associated with better prognostic outcomes (17). In this perspective, the expression of CD8, a cytotoxic T cell surface molecule may bring different properties to the clonal tumor cell in regard of immune dysregulation and immune escape. In our study, we did not observe such a strong relation which may be explained by our limited number of CD8+ CLL patients.

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Conflict of interest: The author declares that there is no conflict of interest regarding the publication of this article.

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