Background. CD20 positive T cells (CD20TCs) have been described in healthy peripheral blood almost twenty years ago. The function of these cells is still unknown. We observed CD20TCs in bone marrow (BM) samples of patients with myelodysplastic syndrome (MDS) and multiple myeloma (MM) at a higher ratio compared to normal blood. In this pilot study we investigated the incidence of CD20TCs amongst lymphocytes in small series of MDS, MM patients and control bone marrow (BM) samples. Furthermore, we used imaging flow cytometry to exclude cell aggregates causing artificial CD20+, CD3+ co-expression. Because its higher incidence in patient samples with malignant disease we assume that CD20TCs may have antitumor immunity. Therefore in CD20TCs we investigated the expression of NKG2D receptor (CD314) which is reported to be important for T cell and NK cell-mediated immunity to tumors.

Methods. BM aspirate samples of 8 MDS, 10 MM patients and 3 controls from patients with acute lymphoblastic leukemia were investigated by flow cytometry. CD20TCs (CD20+, CD3+) were variably conjugated to FITC, PerCP-Cy5.5 and PE-Cy7. In 7 selected cases imaging flow cytometry was done by an Amnis FlowSight instrument (Amnis Corporation, WA, USA) in parallel using the same stained samples.

Results. The mean percentage of CD3+, CD20+ double positive cells in the lymphocytes gate (based on CD45 vs. SSC) was 2.2% in the control BM samples which is similar to the published peripheral blood values. In MDS and MM CD20TC ratios were 16.6% and 16.2%, respectively, which represents 7-fold increase compared to control BM. Imaging flow cytometry capturing the photographs of all events measured showed that CD3+, CD20+ double positive events were real single cells. The ratio of NKG2D positive cells was 65.5% in CD20+, CD3+ and 36.1% in CD20-, CD3+, CD20+ double positive events. Median NKG2D MFI was 1.7x higher in CD20+, CD3+ than in CD20-, CD3+ T cells.

Conclusions. In our study we provide the first direct evidence for the existence of CD20+, CD3+ T cells using morphological pictures by imaging flow cytometry. As the CD20TC ratio was 7-fold higher in malignant conditions in the BM of MDS and MM patients compared to control BM samples we assume that this T cell subset may play a role in antitumor immunity. This is further supported by the fact that the ratio of the NKG2D expressing T cells was 1.8 times higher in CD20TCs than in CD20- T cells and the median fluorescence intensity of NKG2D was 1.7 times higher in CD20+ CD20TCs than in NKG2D-, CD20- T cells. In a prospective study we plan to confirm our preliminary results on higher number of cases and assess the correlation between the prognosis and the ratio of CD20TCs in MDS and MM.


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