G-CSF-primed autologous and allogeneic bone marrow for transplantation in clinical oncology. Cell content and immunological characteristics

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Abstract. 60 samples of G-CSF-primed bone marrow (39 cancer patients and 21 healthy donors) to be used for transplantation to cancer patients were analyzed and compared by main characteristics with historical control and 13 bone marrow samples from control patient with mastopathy. Basing on morphological and multicolor flow cytometry findings certain characteristics of G-CSF-primed bone marrow were discovered, such as a significant increase in blast count in cancer patients as compared to donors and control patients (p<0.037), a higher neutrophil maturation index (p<0.001) and a lower percentage of mature lymphocytes (p<0.008) as compared to the control group. Among lymphocyte populations G-CSF-priming was associated with a significant increase in the total of mature CD3+ T-cells and CD8+ T-killers (p<0.0001) and a decrease in CD56+CD3- and/or CD16+CD3- NK-cells (p<0.006) both in cancer patients and healthy donors in comparison with the controls.

1. Introduction

Allogeneic hemopoietic tissue transplantation alone is a therapy of choice in many patients with hematology malignancies [1, 2, 3, 4].

Bone marrow is preferred for allogeneic transplantation mainly due to lower (4 to 10-fold) content of mature T-cells involved in graft versus host reaction (GVHR) [5 – 9]. Donors’ T-cells though mediate GVHR are needed for adequate recovery of recipient’s immunity functioning and, what is of principal importance, some of their subsets (CD8+ memory T-cells) are mediators of graft versus tumor reaction [10 – 13].

There is a minor regulatory T-cell subset predominating in bone marrow that acts as a protector of post-transplantation GVHR [14, 15], in spite of the fact that most of these cells are mediators of GVHR in allogeneic transplantation of mobilized PB [16].

So, from the immunological standpoint bone marrow is a more attractive material for both auto- and allotransplantations in cancer patients. It is of much importance to study in detail characteristics of
stimulated bone marrow due to high clinical need in this material and equivocal role of some lymphocyte subsets.

The purpose of this study was to analyze in detail morphological and immunological (lymphocyte subset composition) characteristics of granulocyte colony stimulating factor (G-CSF)-primed bone marrow from cancer patients and healthy donors and to compare characteristics of the stimulated bone marrow samples with normal parameters.

2. Materials and methods
We analyzed 39 stimulated bone marrow samples from 39 adult patients with hematology malignancies taken for autologous transplantation and 21 granulocyte colony stimulating factor (G-CSF)-primed bone marrow samples taken from 21 healthy donors to transplant to adult patients with hematology malignancies.

The cancer patient group included 22 females and 17 males, mean age was 38 (range 18 to 57) years. Most patients had Hodgkin’s lymphoma (28) and 11 patients had acute myeloid leukemia (AML). The bone marrow donor group consisted of 10 men and 11 women, mean age was 40 and median age was 37 (range 18 to 61) years.

Both autologous and allogeneic bone marrow samples were taken after G-CSF-priming (at 100 mcg/kg daily) as performed by similar schedules in the patients and donors. The control group was composed of 13 bone marrow aspiration biopsies from females with breast cancer diagnosis excluded.

Each bone marrow sample was characterized by morphology and immunology, analyses were made from the same tube, smears and cells for immunological analysis were prepared directly at the laboratory. Cellularity of samples was counted using a Micros 60 hematology analyzer.

Lymphocyte subset composition and the number of precursor cells were studied immunologically (multicolor, 4- to 8-color flow cytometry) using direct conjugates of monoclonal antibodies to CD34 precursor-cell antigen (Figure 1), and to T-, B- and NK-cell main antigens (CD3, CD4, CD8, CD16, CD56, CD94). Hemopoietic stem cell count was made within all nucleated cells (modified protocol for stem cell count by flow cytometry) [17]. Lymphocyte subsets were counted within the lymphocyte gate (figure 2).

Figure 1. Assessment of CD34+ stem cell count in a donor’s bone marrow sample. A – assessment of proportion of nucleated cells using a syto 16 nucleotropic dye. B – assessment of stem cell (CD34+) count within nucleated (syto16+) cells in the sample.
Figure 2. Cytogram A shows the lymphocyte gate CD45+ (blue). Cytograms B, В, Г, demonstrate lymphocyte subset composition: Figure B – B-cell proportion is 10.83% of lymphocytes (y-axis, upper left quadrant), T-lymphocytes (CD3+) are 62.9% (x-axis), Figure C – CD4+ T-helper proportion is 23.4% (y-axis, upper left quadrant), CD8+ T-killer portion is 39.7% (x-axis, lower right quadrant), Figure D – most (13.2% of lymphocytes) of NK-cells (yellow) are a CD56+CD94+ subset.

3. Results

Comparison of bone marrow morphological composition demonstrated a significantly higher blastosis (p=0.012 and p=0.037) and lower lymphocyte proportion (p=0.017 and p<0.0001) in bone marrow samples from cancer patients than in either donors’ or control bone marrow samples. Neutrophil maturation index was significantly higher in cancer patients as compared to donors’ stimulated bone marrow and control samples (p=0.026 and p<0.0001, respectively).

Mature T-cells (CD3+) were predominant among lymphocytes in stimulated bone marrow with most of them being CD8+ T-killers (table 3).

Comparison of immunological characteristics of bone marrow cells demonstrated predominance of CD34+ precursors in cancer patients’ samples as compared to the donors’ ones though the difference was not significant. The largest number of CD34+ cells (10.9%) was found in AML cases, and the number of CD34+ cells was proportional to blast count (p<0.0001) in cancer patients, while no such correlation was seen in the donors’ group.

Stimulated bone marrow samples from cancer patients showed a significant predominance in percentage of CD3 lymphocytes (p=0.04), CD8+ T-killers (p<0.0001) and accordingly lower immunity regulating index (p<0.0001) as compared to donors’ samples.

Stimulated bone marrow samples from cancer patients demonstrated a reverse relationship between immunity regulating index and neutrophil maturation index (R=- 0.363, p=0.027), this correlation in the donors’ group was not significant.

Comparison of lymphocyte subset composition in bone marrow samples from HL versus AML subgroups showed a significant predominance of CD3+ lymphocytes (82.9±3.3% vs. 68.3±4.5%; p=0.021) and CD4 leukocytes (2.14±0.2% vs. 1.02±0.16%; p=0.001) in the AML samples.

Samples of stimulated bone marrow from HL patients were characterized by a significantly higher content of CD56+ NK-cells (5.6±0.5% vs. 3.5±0.5%, p=0.05) and a higher proportion of CD19+ B-lymphocytes (26.4±5.9% vs. 8.1±2.9%, p=0.01) as compared to AML subgroup.

Comparison of immunological characteristics of stimulated bone marrow samples (from both cancer patients and donors) with those from the controls demonstrated a significantly higher proportion of T-killers and the lowest immunity regulation index in samples from cancer patients as compared with the controls (p=0.0001). Stimulated bone marrow lymphocytes from both cancer patients and donors contained a significantly higher proportion of CD16+ and CD56+ NK-cell subsets (p<0.06 and p<0.004, respectively) as compared with the control group.
4. Conclusion
Our study discovered statistically significant differences in morphological and immunological (lymphocyte subsets) compositions of G-CSF-primed bone marrow as increase in blasts, predominance of younger granulocytes due to promyelocytes and myelocytes, increased proportion of mature erythroblasts among erythroid cells and decreased proportion of mature lymphocytes in samples from both cancer patients and donors. As concerns lymphocyte subsets, G-CSF-priming was associated with predominance of the total of mature CD3+ T-cells and CD8+ T-killers and decreased number of CD56+CD3- and/or CD16+CD3- NK-cell subsets.

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