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The frequency of myeloid and lymphoid dendritic cells in multiple myeloma patients is inversely correlated with disease progression*

Ocena subpopulacji mieloidalnych i limfoidalnych komórek dendrytycznych u chorych na szpiczaka mnogiego w różnych stadiach zaawansowania choroby

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Summary

Multiple myeloma (MM) is a neoplastic disease characterized by proliferation and prolonged survival of clonal plasma cells, most frequently occurring in the bone marrow, but also in other tissues. Dendritic cells (DCs) are a heterogeneous population of leukocytes defined as professional antigen presenting cells playing a key role in anticancer immunity. The purpose of this study was to evaluate subpopulations of myeloid and lymphoid DCs in the peripheral blood (PB) and bone marrow (BM) of patients with MM in the different clinical stages of MM and in correlation with known prognostic factors. The study involved 50 patients diagnosed with MM before the initiation of anticancer therapy and 25 individuals belonging to the control group. The mean percentage of myeloid and lymphoid DCs was determined using flow cytometry. In the present study, we demonstrated a significant reduction in the percentages of both myeloid and lymphoid DCs in MM patients, more pronounced in those with the worse prognosis as determined by the high levels of β2 microglobulin. Accordingly, a marked decrease in the proportions of both myeloid and lymphoid DCs in the BM of patients with advanced clinical stage (III) compared to earlier stages (I+II) was also found. Our results suggest that the degree of DC subpopulations deficit could be related to the MM progression, which in consequence may contribute to the MM-related impairment of the immune responses.

Key words: multiple myeloma • myeloid dendritic cells • lymphoid dendritic cells • β2 microglobulin

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Introduction

Multiple myeloma (MM) is a neoplastic disease characterized by proliferation and prolonged survival of clonal plasma cells, most frequently occurring in the bone marrow, but also in other tissues. Neoplastic plasmocytes usually produce one type of immunoglobulin – a monoclonal protein (M protein). Multiple myeloma accounts for approximately 1% of all cancers and 14% of hematological malignancies. It usually affects older people, especially men. Symptoms of the disease include bone destruction with pathological fractures, kidney failure and frequent infections, which are among the major causes of patient mortality [1,2].

There are two main staging systems to determine the disease stage: the Durie-Salmon staging system and the International Staging System (ISS). The Durie-Salmon clinical staging system incorporates several factors correlating with tumor cell mass. Using this method, stage is determined based upon a subjective measure of tumor cell density in the bone marrow along with measures of end organ damage (renal insufficiency, anemia, hypercalcemia, lytic bone lesions) and immunoglobulin burden [8]. ISS is based on the levels of serum beta-2 (b2) microglobulin and serum albumin, and divides the disease into three stages with prognostic significance [10]. The serum b2 microglobulin level is elevated (i.e., >2.7 mg/L) in 75% of patients at the time of diagnosis. Patients with high values have inferior survival [25].

Multiple myeloma is associated with immunosuppression that is responsible for both the impaired anti-tumor response and the aforementioned frequent and recurrent infections. It was primarily found that immune defects in MM patients included abnormalities in the number and function of B, T and NK cells [14]. More recent data revealed convincing evidence on the important role of dendritic cells (DCs) in the impairment of the immune system [26].

Dendritic cells are a heterogeneous population of leukocytes defined as professional antigen presenting cells. They are capable of presenting both exogenous antigens as well as autoantigens. DCs perform a fundamental role in the activation of the immune responses, both innate and specific. Dendritic cells express on their surface the following antigens: CD1a, CD40, CD54, CD58, CD80, CD83, CD86, DC-SIGN. They are characterized by high expression of MHC class I and II [3,5,13]. There are two major subpopulations of DCs: myeloid and lymphoid (plasmacytoid). DCs occur in almost all tissues of the body. In respect of anatomic location, maturity and activation state they are morphologically and functionally divergent [2,5,19,28]. Lymphoid DCs stimulate the differentiation of Th0 lymphocytes into Th2, thus inducing a humoral response [15]. Myeloid DCs direct differentiation of Th0 cells towards Th1 and Th17, and induce a cellular response [17]. It should be noted that lymphoid DCs secrete INF-α and IFN-β, which affect the activation of T CD8+ and NK cells [23]. For this reason, it is difficult to distinguish between the functions of myeloid and lymphoid dendritic cells [13]. The degree of DCs' maturity is also important for their functions. Mature DCs induce an immune response, whereas the immature cells may lead to the development of tolerance to specific antigens [16]. Factors that inhibit maturation of DCs include cytokines, such as IL-10 secreted by tumor cells [21]. Also IL-6 and VEGF, high serum levels of which are observed in patients with multiple myeloma, inhibit differentiation and maturation of dendritic cells [9,12,22].

The purpose of this study was to evaluate subpopulations of myeloid (BDCA-1+) and lymphoid (BDCA-2+) DCs in the PB and BM of patients with MM in different clinical stages and in correlation with known prognostic factors: serum b2 microglobulin level and albumin concentration.

Material and methods

Study and control group

The study involved 50 patients diagnosed with MM at the Department of Hematology in the Holycross Cancer Centre in Kielce. The research samples were collected after the MM diagnosis before the initiation of anticancer therapy.

The average age of patients was 64 (ranging from 34 to 81). In the study group, there were 27 women and 23 men.

The diagnosis of multiple myeloma was based on the criteria of the International Myeloma Working Group. At the time of diagnosis patients were staged according to the Durie-Salmon classification, as follows: stage I – 14 patients, stage II – 8 patients and stage III – 28 patients. The research protocol was approved by the Ethics Committee of the Medical University of Lublin and all patients gave written informed consent.

Patients with the coexistence of other chronic diseases, such as allergic diseases, autoimmune diseases, other cancer diseases, serious infections, as well as those treated with immunosuppressants and immunomo-

dulators or receiving transfusion of blood products in the past 6 months, were excluded from the study. The control group consisted of 25 subjects (15 women and 10 men) undergoing hip replacement surgery due to osteoarthritis, from whom bone marrow and peripheral blood samples were collected at the time of surgery. The median age of subjects in the control group was 63.7 (ranging from 33 to 79).

Cytometric analysis of myeloid and lymphoid DCs

Peripheral blood and bone marrow samples were collected into heparinized tubes and immediately processed. Peripheral blood and bone marrow mononuclear cells (PBMCs and BMMCs, respectively) were separated by density gradient centrifugation. After isolation, the cells were surface-labeled with the following monoclonal antibodies (MoAbs) conjugated with relevant fluorochromes: anti-CD45/FITC and anti-CD14/RPE (in order to determine precisely the goal for a population of mononuclear cells), anti-BDCA-1/FITC and anti-CD19/PE-Cy5 (to estimate the percentage of immature myeloid DCs), and anti-BDCA-2/FITC and anti-CD123/RPE (to determine the frequency of immature lymphoid DCs). PBMCs and BMMCs with the phenotype BDCA-1+CD19- were considered to be myeloid DCs, whereas those presenting phenotype BDCA2+CD123+ were assigned to lymphoid DCs. Labeled cells were washed and analyzed with the flow cytometer (BD FACSCalibur). In each case, staining was compared with that of the appropriately labeled isotype control antibody.

Statistical analysis

Statistical analysis of the results was conducted using Statistica 9.0. Deviation from normality was evaluated by Kolmogorov–Smirnov test. Data were expressed as the mean value ± SD, median, minimum and maximum. Differences between groups were assessed using the Mann–Whitney *U* test. Pearson or Spearman correlation analyses were used to analyze the correlation. A value *p* less than 0.05 was considered statistically significant.

Results

Assessment of the DC subsets in the peripheral blood (PB)

In PB, the value of the mean percentage of myeloid DCs of MM patients was 0.15±0.13% (median 0.13%, min. 0.02%, max. 0.63%), and was significantly lower than corresponding cells in the control group – 0.55±0.22% (median 0.55%, min. 0.18%, max. 1.00%) (p=0.000001) (Figure 1). Similarly, we found the mean frequency of lymphoid DCs of MM patients markedly diminished compared to that observed in the control group – 0.13±0.13% (median 0.11%, min. 0.01%, max. 0.6%) vs. 0.4±0.25% (median 0.37%, min. 0.15%, max. 1.04%) (p=0.00005) (Figure 2).

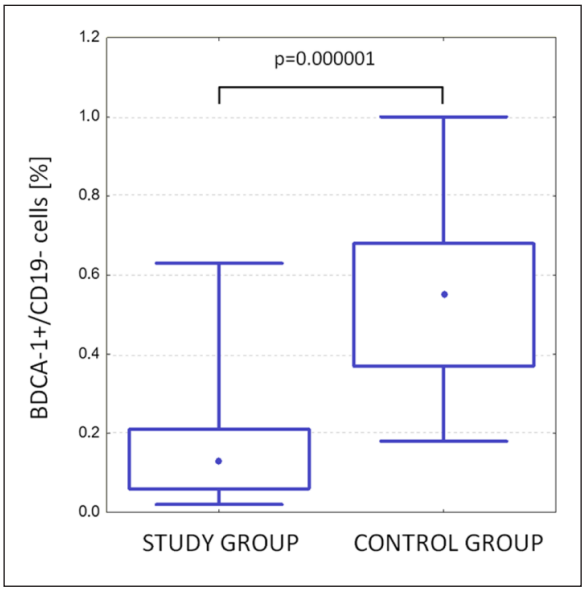

Fig. 1. Median percentage of myeloid DCs in the PB of MM patients and control subjects

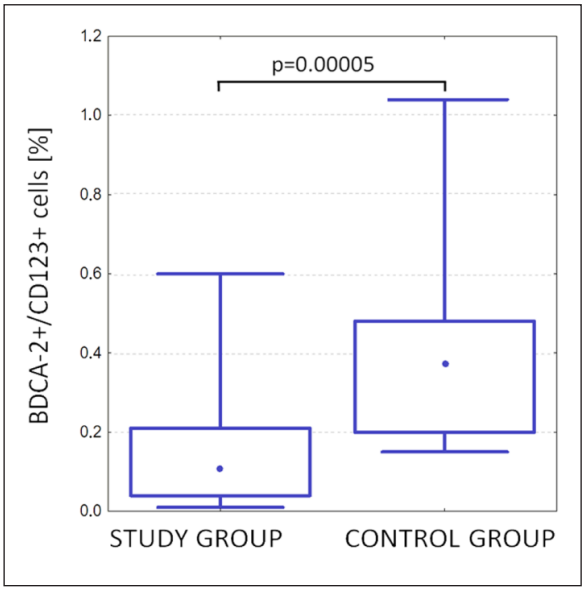

Fig. 2. Median percentage of lymphoid DCs in the PB of MM patients and control subjects

Assessment of DC subsets in bone marrow (BM)

The mean percentage of BM myeloid DCs from MM patients was significantly lower compared to corresponding cells isolated from the control group – 0.12±0.09% (median 0.1%, min. 0.01%, max. 0.54%) vs. 0.27±0.09% (median 0.25%, min. 0.16, max. 0.4) (p=0.000009) (Figure 3). Similarly, the mean proportion of lymphoid DCs isolated from the BM of MM patients was markedly lower compared to those isolated from the control group – 0.23±0.22% (median 0.15%, min. 0.02%, max. 0.84%) vs. 0.9±0.88% (median 0.53%, min. 0.2%, max. 3.57%) (p=0.00005) (Figure 4).

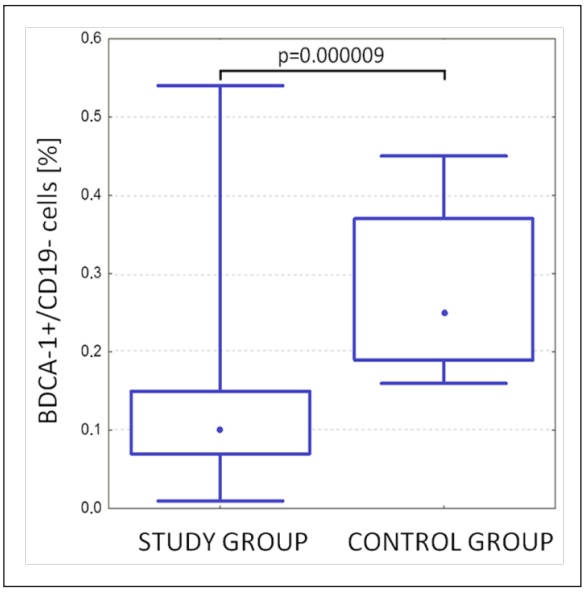

Fig. 3. Median percentage of myeloid DCs in the BM of MM patients and control subjects

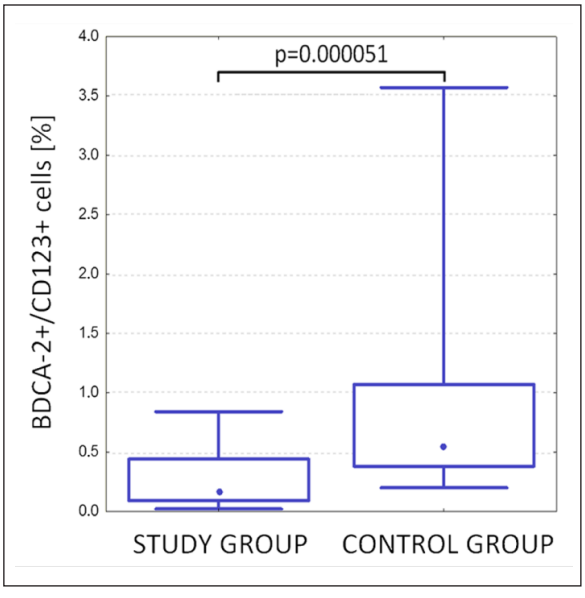

Fig. 4. Median percentage of lymphoid DCs in the BM of MM patients and control subjects

Correlations between DCs subsets and prognostic factors

Assessment of DC subsets in relation to serum concentration of β2 microglobulin in MM patients

There was a significant inverse correlation between β2 microglobulin serum concentrations and the percentages of myeloid (p=0.002, r=-0.5) as well as lymphoid (p=0.003, r=-0.48) DCs in patients' PB (Figure 5 and Figure 6, respectively). In BM, such a negative correlation was observed only when we evaluated the frequencies of myeloid DCs in the context of serum levels of this prognostic factor (p=0.0004, r=-0.56) (Figure 7).

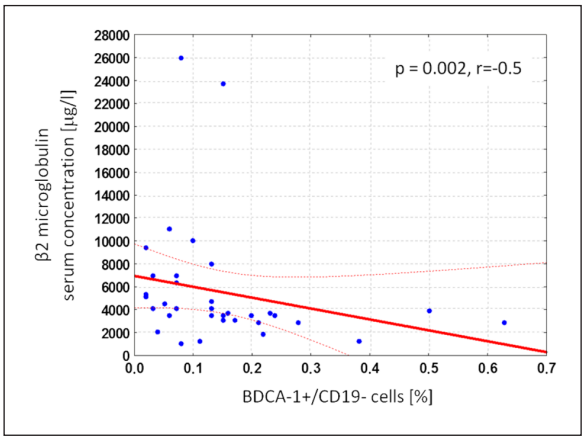

Fig. 5. The correlation between the percentages of myeloid DCs in PB of MM patients and serum levels of β2 microglobulin

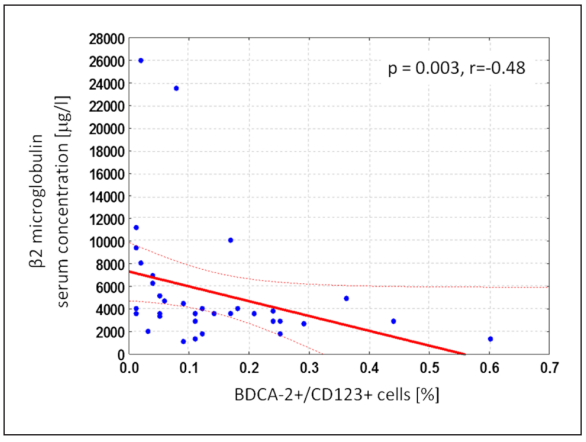

Fig. 6. The correlation between the percentages of lymphoid DCs in the PB of MM patients and serum levels of β2 microglobulin

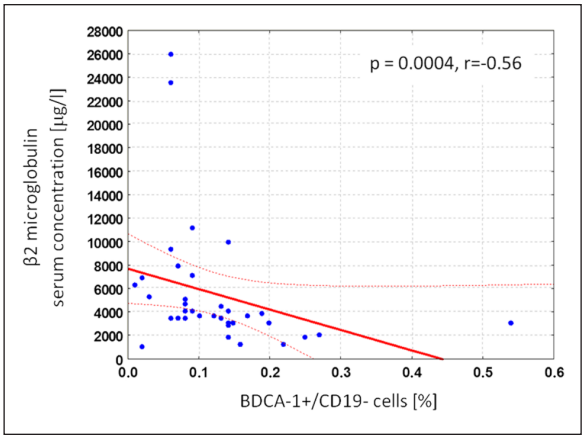

Fig. 7. The correlation between the percentages of myeloid DCs in the BM of MM patients and serum concentration of β2 microglobulin

Assessment of DC subsets in relation to albumin concentrations in sera of MM patients

Although in the group of patients with less advanced MM (stage I+II) the value of the mean albumin concentration

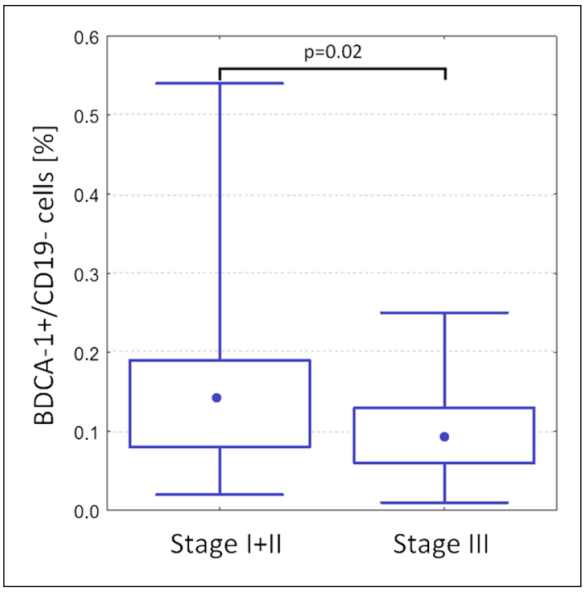

Fig. 8. Median percentage of myeloid DCs in the BM of MM patients in stage I and II compared to stage III according to Durie-Salmon staging system

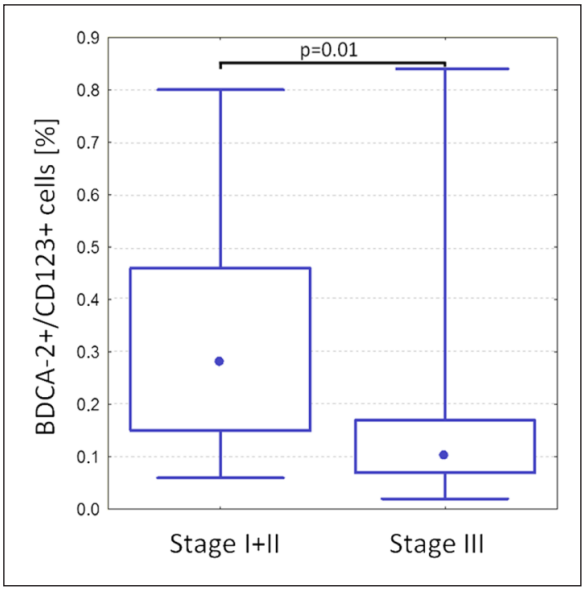

Fig. 9. Median percentage of lymphoid DCs in the BM of MM patients in stage I and II compared to stage III according to Durie-Salmon staging system

was 3.58 g/L (median 3.49 g/L, min. 2.32 g/L, max. 4.97 g/L), and was lower than in patients with more advanced MM (mean 4.16 g/L, median 4.14 g/L, min. 3.59 g/L, max. 4.72 g/L), the difference was only of borderline significance (p=0.06). Nevertheless, we did not find any statistically significant correlations between the percentages of DC subsets and albumin concentrations either in the PB or in the BM.

Correlation of DC subsets with clinical stage of MM

We found that only frequencies of DCs isolated from BM correlated with the clinical stage of MM. In particular, the mean proportion of myeloid DCs from BM of patients in stage III (according to the Durie-Salmon classification) was significantly lower in comparison with the corresponding cells of patients in stages I and II – 0.09±0.042% (median 0.098%, min. 0.064%, max. 0.14%) vs. 0.15±0.83% (median 0.17%, min. 0.09%, max. 0.196%) (p=0.02) (Figure 8). A similar dependence concerning the mean percentage of lymphoid DCs in BM was observed – 0.12±0.08% (median 0.1%, min. 0.07%, max. 0.18%) vs. 0.32±0.93% (median 0.29%, min. 0.16%, max. 0.46%) (p=0.01) (Figure 9).

Discussion

Disorders of the immune system play an important role both in the pathogenesis and clinical course of MM, being responsible not only for the increased incidence of infections, but also for the disease progression. Dendritic cells constitute one of the key populations in the immune system. In patients with solid tumors as well as with hematological malignancies, many abnormalities in the number and function of DCs were described. However, still little is known about the changes in DC subpopulations in patients with MM.

In the present study we found significantly reduced percentages of myeloid and lymphoid DCs in both PB and BM of MM patients compared to the healthy subjects. Similar results concerning a marked decrease in the percentages of both DC subpopulations in the PB of MM patients were described by Kovarova et al. [18], Do et al. [7], and Martin-Ayuso et al. [20]. Of note, the latter authors found that the amount of DC precursors in the PB of MM patients was unchanged [20]. It should be emphasized, however, that data on the evaluation of DC subpopulations in the BM of MM patients are still lacking.

There are many potential explanations for the reduction in the percentages of DCs in the PB and BM of patients with MM. It has been shown that IL-6 inhibits the formation of colonies of DC progenitor cells and DC maturation [24] Another factor adversely affecting the formation and maturation of dendritic cells is VEGF [4]. It has been shown on animal models as well as in clinical observation that a high concentration of VEGF reduces the percentage of circulating peripheral blood DCs, and impairs their functions [9]. Yang et al. demonstrated that neutralization of VEGF restores the normal function of DCs in patients with MM [30].

Next, we found a significant inverse correlation between serum concentrations of β2 microglobulin of MM patients and the proportions of both myeloid and lymphoid DCs in the PB as well as with the frequencies of myeloid DCs in the BM. Since β2 microglobulin is one of the most important negative prognostic factors in MM correlating with disease activity [6,25], the lower percentages of both DC subpopulations seen in patients with higher concentration of β2 microglobulin might reflect greater disorders in the immune system in more aggressive course of the disease. To date, there are only a few reports

on the relationship between the frequency of DCs and the serum concentration of β2 microglobulin. Xie et al. observed an inhibitory effect of β2 microglobulin on DCs and their capacity for T cell stimulation [29]. On the other hand, Wang et al. demonstrated that the bone marrow microenvironment occupied by MM cells might affect DCs as well [27]. They also demonstrated that the addition of antibodies against IL-6, IL-10 and TGF-β to a culture containing DC colonies and particular factors of BM microenvironment could partly restore the function of DCs [27]. Thus, it seems that both the MM cells and the increased serum concentration of β2 microglobulin might disturb the subpopulations of DCs either in the BM or PB.

Additionally, we assessed DC subpopulations in PB and BM of patients in different clinical stages according to the Durie-Salmon classification. Although a more pronounced decrease in both studied DC subsets was noted in the PB from patients with the most advanced disease, the differences did not reach statistical significance, when compared to earlier stages. Our observation is consistent with the previous data published by Ratta et al. [24]. In turn, Harrison et al. found that disorders of DCs in patients with monoclonal gammopathy of undetermined significance (MGUS) are extremely rare, whereas in MM patients these changes are frequently observed [12]. This suggests that the progression of MGUS to MM might correspond with the increasing adverse influence of MM cells on DC subpopulations. In the present study, we originally found that the frequency of both myeloid and lymphoid DCs in the BM was significantly lower in the patients in stage III compared to the patients in the less advanced disease, which may confirm the previous report by Wang et al., suggesting a direct adverse effect of the BM microenvironment on DCs [27].

In summary, herein we demonstrated the reduction in the percentages of both myeloid and lymphoid DCs in MM more pronounced in those with the worse prognosis as determined by higher levels of β2 microglobulin. This observation may reflect more severe disorders in DC populations in patients with more active disease. It also seems that factors secreted by the MM plasmocytes or tumor microenvironment negatively affect the DCs, which is manifested by a decrease in the frequency of both myeloid and lymphoid DCs in the BM isolated from patients at an advanced clinical stage in comparison with patients at earlier stages. The ultimate role played by DCs in the development of MM is still unexplained and requires further studies.

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The authors have no potential conflicts of interest to declare.