(MSCs), stromal adult stem cells with immunomodulatory properties. The aim of this study was to investigate the role of MSCs on expansion and activation of G-MDSCs. Using flow cytometric analysis, we observed that G-MDSC (CD11b+CD33+CD14-HLADR-) percentage in MM was greater than MGUS and healthy donors (HD) (61.2±1.6% *versus* 53.7±2.2% and 49.7±1.4% respectively, p=0.026 and p<0.001). We found a T-cell anergy driven by MM-G-MDSCs. Subsequently, we investigated the capacity of MSCs from MGUS and MM patients and HD to generate MDSCs. Briefly, human peripheral blood mononucleated cells isolated from HD were cultured alone, with HD (n=6), MGUS (n=6) or MM MSCs (n=7) (1:100 ratio). After one week, PBMCs were collected and G-MDSCs isolated using anti-CD66b magnetic microbeads. The phenotype of G-MDSC was confirmed by cytofluorimetric analysis. Their immunosuppressive capacity was analyzed evaluating T-cell proliferation when co-cultured with autologous CFSE-labeled T cells stimulated by phytohaemagglutinin (PHA). Only MM MSCs-educated G-MDSC exhibited suppressor effect with a reduction of T cell proliferation of about 34±9.6% (p<0.01) compared to G-MDSC control (isolated after culture in medium alone). Notably, neither MDSCs control nor myeloid cells co-cultured with HD or MGUS MSCs showed suppressive activity. Using real time PCR, we analyzed the expression of immune modulatory factors (Arg1, NOS2, COX2, TNF, TGF, IL6, IL10, IL1) by MSCs after 48h of co-culture with PBMCs. MM MSCs showed an increase of COX2, IL6, IL10, IL1, TGF and NOS2 expression (p<0.05) compared to HD MSCs, suggesting that multiple mechanisms are involved in MDSCs induction by MM MSCs. The same immune modulatory factors were investigated in MDSCs before incubation with T cells. MM MSCs-educated MDSCs expressed higher levels of Arg1, NOS2 and IL6 (p<0.05) compared to MDSCs control. In conclusion, MSCs from MM but not MGUS patients are able to activate G-MDSCs with potential implication in immune escape that favours plasma-cells growth, survival and resistance to drugs.

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BORTEZOMIB INHIBITS OSTEOCLASTOGENESIS AND BONE RESORPTION THROUGH MODULATION OF CHIT1 AND YKL40 EXPRESSION

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Osteolytic bone disease is a common manifestation of multiple myeloma (MM) that leads to progressive skeleton destruction and is the most severe cause of morbidity in MM patients. CHIT1 and YKL40 are mammalian chitinases evolved to hydrolyze chitin. Recently, our group has demonstrated that chitinases are important during human OC differentiation and silencing CHIT1 and YKL40 with siRNAs in mature osteoclasts (OCs) results in decreased bone resorptive activity *in vitro*, suggesting a key role for chitinases in OC function. The aim of this work was to investigate the inhibition of osteoclastogenesis by bortezomib (BO) analyzing its regulation of chitinase expression and explore the role of chitinases in osteolytic activity of plasma cells (PCs). First, we confirmed that BO exposure during OC differentiation led to the inhibition of osteoclastogenesis reducing expression of OC markers (MMP9, RANK, CTSK and TRAP; p<0.0001) and reducing bone digestion ability in a dose-dependent manner (2,5 and 5 nM). We further showed that drug treatment down-regulated expression of CHIT1 and YKL40 in a dose-dependent manner (p<0.0001). In particular, BO affected both the component (cytoplasmic and secretory) of CHIT1, YKL40 and MMP9, whose expression is closely associated with chitinases production. Measuring CHIT1 enzyme activity in cell-free supernatants, it also resulted affected by BO during all differentiation process (70±5%, 62±1%, 65±4% and 68±4% respectively at 5, 7, 15 and 21 days; p<0.002). Moreover, immunofluorescence evaluation of OCs showed that BO was able to translocate YKL40 into the nucleus, while CHIT1 remained into the cytoplasm. Subsequently, we chose to investigate if chitinases are involved in PC ability to participate in bone resorption. U266 expressed higher CHIT1 activity (466±111.29 *vs* 216±19.07 and 98±41.44) and showed higher levels of CHIT1 and YKL40 mRNA than SKM-M1 and MM1 MM cell lines (p<0.001). A significant reduction of MM cell digestion activity was observed silencing CHIT1 and YKL40 alone (21.98±5.2% and 25.08±3% *vs* negative control; p<0.003) or in combination (82.85±7%; p<0.0001). Interestingly, exposure of U266 to BO was able to decrease CHIT1 and YKL40 expression in a dose-dependent manner (p<0.0001). In conclusion, we demonstrate that BO treatment inhibits osteoclastogenesis and bone resorption through downregulation of CHIT1 and YKL40, not only in OCs but also in PCs. All these BO effects contribute to its antimyeloma efficacy.

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AZACITIDINE IMPROVES THE T-CELL REPERTOIRE IN PATIENTS WITH MYELODYPSLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA WITH MULTILINEAGE DYSPLASIA

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Patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) with multilineage dysplasia show several immunological abnormalities. Azacitidine represents a therapeutic option for these disorders and has been demonstrated to potentially influence T-cell polarization. The aim of this study is to monitor the kinetic of the T-cell receptor (TCR) repertoire during Azacitidine treatment in order to explore its potential ability to reverse the immune derangement typical of these patients. Our study consisted in a flow cytometric and spectratyping analysis performed on the peripheral blood of 11 patients and 30 normal controls. The flow cytometry analysis was based on a panel of 24 β variable (BV) family-specific antibodies. The profile of the third complementarity-determining-region (CDR3) in separated CD4+ and CD8+ T-cells was then analyzed by spectratyping. By flow cytometry, we first demonstrated an improvement from baseline to the following evaluations in the frequency of CD3+ (mean 63.00% *vs* 68.24%, p=0.03), CD4+ (40.09% *vs* 42.05%, p=0.04) and CD8+ cells (20.27% *vs* 25.10%, p=0.02), while the frequency of regulatory T-cells (0.53% *vs* 0.45%, p=0.84) and BV expansions was stable in both CD4+ (mean 1.60% *vs* 2.53%, p=0.36) and CD8+ cells (5.20% *vs* 5.60%, p=0.79). Noteworthy, when monitored by spectratyping during their treatment our patients showed significant changes in their CDR3 profiles, which were much more evident in CD4+ T-cells. In fact, the frequency of skewed BVs was significantly decreased from baseline to the following evaluations in the CD4+ subset (mean 81.45% *vs* 70.17%, p=0.004). This pattern was even more pronounced in patients responding to Azacitidine (89.60% *vs* 61.47%, p=0.002). Also in the CD8+ subset a slight but statistically significant trend towards a reduction in the frequency of skewed CDR3 profiles (mean 99.27% *vs* 98.74%, p=0.01) was demonstrated. Our findings firstly confirmed in our patients an overall derangement of the TCR repertoire. However this pattern seems to gradually improve during Azacitidine treatment, as witnessed by the improvement in T-cell counts observed on flow cytometry but much more by the progressive restoration of the CDR3 diversity detected by spectratyping, especially within the CD4+ subset. Therefore our data suggest that Azacitidine could be potentially able to reverse the immune derangement typical of patients with MDS and AML with multilineage dysplasia.