Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome

Astrid Olsnes Kittang1,2,*, Shahram Kordasti1,3, Kristoffer Evebø Sand2, Benedetta Costantini1, Anne Marijn Kramer1, Pilar Perezabellan1, Thomas Seidl1, Kristin Paulsen Rye2, Karen Marie Hagen2, Austin Kulasekararaj1, Øystein Bruserud3, and Ghulam J. Mufti1

1Department of Haematological Medicine; King’s College London and King’s College Hospital; London, UK; 2Department of Clinical Science; Faculty of Medicine and Dentistry; University of Bergen; Bergen, Norway

*Correspondence to: Astrid Olsnes Kittang; Email: astrid.kittang@uib.no

Submitted: 02/05/2015; Revised: 06/07/2015; Accepted: 06/08/2015

Keywords: CD4+ T cells, CX3CR1, CXCR4, cytokines, flow cytometry, immune-surveillance, AML, MDS, MDSCs, Tregs

Although the role of CD4+ T cells and in particular Tregs and Th17 cells is established in myelodysplastic syndrome (MDS), the contribution of other components of immune system is yet to be elucidated fully. In this study we investigated the number and function of myeloid derived suppressor cells (MDSCs) in fresh peripheral blood and matched bone marrow samples from 42 MDS patients and the potential correlation with risk of disease progression to acute myeloid leukemia (AML). In peripheral blood, very low-/low risk patients had significantly lower median MDSC number (0.16×109/L(0.03–0.40)) compared to intermediate-/high-/very high risk patients, in whom median MDSC counts was 0.52×109/L(0.10–1.78), p < 0.005. When co-cultured with CD4+ effector T-cells (T-effectors), MDSCs suppress Teffector proliferation in both allogeneic and autologous settings. There was a positive correlation between the number of Tregs and MDSCs (Spearman R = 0.825, p < 0.005) in high risk and not low risk patients. We also investigated MDSCs’ expression of bone marrow-homing chemokine receptors, and our data shows that MDSCs from MDS patients express both CXCR4 and CX3CR1 which might facilitate migration of MDSCs to bone marrow. Monocytic MDSCs(M-MDSCs) which are more frequent in the peripheral blood express higher levels of CX3CR1 and CXCR4 than the granulocytic subtype (G-MDSCs), and circulating M-MDSCs had significantly higher CX3CR1 expression compared to bone-marrow M-MDSCs in intermediate-/high-/very high risk MDS. Our results suggest that MDSCs contribute significantly to the dysregulation of immune surveillance in MDS, which is different between low and high risk disease. It further points at mechanisms of MDSCs recruitment and contribution to the bone marrow microenvironment.

Introduction

MDS is a group of diseases with clonal proliferation of dysplastic bone marrow haematopoietic cells. Recent studies have established that T cell mediated immune dysregulation is an important feature of MDS. The “immune signature” is substantially different in low and high risk MDS. While the low risk disease is characterized by the expansion of pro-inflammatory T cells (in particular Th17), the expansion of T regulatory cells (Tregs) is the main feature of high risk disease. However, the mechanism of this immune-signature switch is not fully understood.1 Myeloid derived suppressor cells (MDSCs) are a group of immature myeloid cells with a potent immunosuppressive effect which are expanded in an inflammatory environment.2 The role of MDSCs in suppressing immune mediated tumor surveillance and T cell polarization has been demonstrated in solid tumors.3 It has also been shown that MDSCs not only could induce myelodysplasia4 but also that targeting them by NK cells may be therapeutically beneficial in MDS.5 However, it is unclear whether MDSCs play a similar role in MDS as in solid tumors in transforming the inflammatory environment in low risk disease to a more inhibitory environment in high risk MDS.

Two major subsets of MDSCs have been described; Granulocytic (G)-MDSC and monocytic (M)-MDSC, and the frequency of the subsets vary between different tissues.6 In humans, MDSCs have a phenotype that is lineage negative and the cells

© Astrid Olsnes Kittang, Shahram Kordasti, Kristoffer Evebø Sand, Benedetta Costantini, Anne Marijn Kramer, Pilar Perezabellan, Thomas Seidl, Kristin Paulsen Rye, Karen Marie Hagen, Austin Kulasekararaj, Øystein Bruserud, and Ghulam J. Mufti

This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc-3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.
are usually CD11b+, CD33+ & HLA-DR neg/low.2 MDSCs have a key role in immune response regulation and the plasticity of CD4+ T cells. MDSCs produce Interleukin (IL)-10, Transforming Growth Factor (TGF)-β, arginase 1 (ARG1) and inducible nitric oxide synthase (iNOS2), suppress T cells by depriving them of arginine and cysteine and are capable of inducing Tregs.9–9 G-MDSC can in addition produce reactive oxygen species (ROS). MDSC depletion in tumor-bearing mice inhibits tumor progression and metastasis, suggesting that these cells have a key role in tumor specific immune response.10–12 Although the increased number of MDSCs in MDS has been shown,4 it is not clear whether this expansion is MDS subtype specific and which subsets of MDSCs are expanded. The effect of MDSCs on T cells function, in particular CD4+ T effectors and Tregs, in MDS has not been studied previously.

Migration of MDSCs to cancer environment is mediated by the CXCR4/CXCL12-axis.13 The CXCR4-CXCL12 interaction is important for keeping the hematopoietic stem cells in their niches,14,15 and bone marrow plasma from MDS patients shows elevated CXCL12 levels.16 CX3CR1 binds to chemokine and adhesion molecule CX3CL1, a transmembrane cleavable protein whose release is induced by IFNγ and TNF-α,17 cytokines present in low risk MDS bone marrow.18,19 The aims of this study were to investigate the MDSCs number, distribution and function, correlation with the number of Tregs, their bone marrow homing chemokine receptor expression and the correlation with the risk of progression to AML.

Results

Higher numbers of MDSCs in high risk MDS patients

Bone marrow milieu in MDS patients evolves from a proinflammatory environment in low risk patients into an immunosuppressive one in high risk MDS. To assess the potential role of MDSCs in this shift, we compared MDSC frequency and absolute number in MDS patients within different IPSS-R categories; Very Low Risk (VLR)/Low Risk (LR) compared to Intermediate Risk (INT)/High Risk (HR)/Very High Risk (VHR) MDS. Total MDSCs were defined as lineage−/CD11b+/CD33+ cells.

In peripheral blood from VLR/LR categories median MDSC numbers (defined as lineage−/CD11b+/CD33+, see Fig. 4) were significantly lower (0.16 × 10⁹/L (0.03–0.40)) compared to INT+HR+VHR patients, in whom median MDSC counts were 0.52 × 10⁹/L (0.10–3.92), p < 0.005 (Fig. 1A). Peripheral blood MDSCs were higher in VLR + LR MDS compared to healthy age matched donors (median 2.53 % (0.3–10.1) vs. 1.03 % (0.40–2.39), p < 0.0003, and higher in INT + HR + VHR patients (median 5.87 % (2.13–40.90)) compared to VLR + LR patients (p = 0.0006) and healthy donors (p = 0.040) (Fig. 1B). The same difference was found when comparing MDS patients based on WHO classification. Patients with bone marrow blasts 5 % or more (RAEB) had higher absolute counts 0.38×10⁹/L (0.10 – 3.92 × 10⁹) vs. patients with less than 5 % bone marrow blasts (RC/RARS/RCMD/MDS-U) (0.16 × 10⁹/L (0.03–0.40 × 10⁹)), p < 0.01, (Fig. 1C). MDSC percentages were higher in RAEB, median 3.55 (2.13 – 40.90) compared to (RC, RARS, RCMD, MDS-U, median 1.43 (0.30–10.10)) p = 0.0151, as well as healthy donors (median 1.03 (0.40–2.39)) p < 0.0001. Patients with less than 5 % bone marrow blasts also had higher MDSC percentages compared to healthy donors, p = 0.0015. (Fig. 1D).

In RAEB patients there was a positive correlation between the absolute numbers of MDSC and Tregs in peripheral blood (Spearman R = 0.825, p < 0.005) which was not the case in patients with less than 5 % bone marrow blasts (Fig. S1A).

G-MDSC subset is expanded in high risk MDS

G-MDSCs are a subtype of MDSCs which are phenotypically and functionally different from M-MDSCs.20 G-MDSCs were defined as lineage−/CD11b+/CD33+/CD15+. G-MDSCs were increased (frequency and absolute numbers) in INT+HR+VHR disease (median 0.88 % (0.3–10.8) (median 0.31 × 10⁹/L) compared to VLR+LR disease (median 0.22 % (0.02–8.43) (median 0.011 × 10⁹/L); p < 0.01 (p = 0.0001) and to healthy donors (median 0.2 % (0.02–1.6)); p = 0.02 (Fig. 1E and Fig. 1F). This difference was also significant when comparing RAEB versus RC/RARS/RCMD/MDS-U patients (median 0.13 vs. 0.01, p < 0.01) (Fig. S1B).

In high- and low risk MDS patients, M-MDSCs were higher in peripheral blood (median 0.52% (0.06–6.3)) compared to bone marrow (median 0.27% (0.002–1.29), p < 0.02, (Fig. S1C) and the same pattern was seen in age-matched healthy donors (median bone marrow-M-MDSC 0.1 % (0.1–0.4), data not shown.

G- and M-MDSC subsets were positive for intracellular IL-10 and TGF-β. Both IL-10 and TGF-β levels were higher in G-MDSCs compared to M-MDSCs (relative median fluorescence intensity (R-MFI) 7.9 (2.2–48.4) vs. 3.8 (0.8–10.5), p = 0.003 for IL-10 and R-MFI 15.0 (1.4–30.4) vs. 4.8 (0.4–12.4), p = 0.02 for TGF-β) (Fig. 2A and Fig. S1D and E).

Chemokine receptor expression on MDS-MDSCs

We hypothesized that immunosuppression mediated by MDSCs protects the bone marrow MDSC clone. The next step therefore was to investigate bone marrow homing receptor expression on circulating MDSCs. In MDS the G-MDSCs and M-MDSCs show different chemokine receptor expression patterns. CXCR4 is responsible for homing to bone marrow and stem-cell niches,21 while CX3CR1 is involved in bone marrow metastasis.22

M-MDSCs CX3CR1 expression was significantly higher in peripheral blood from intermediate and high risk MDS patients compared to bone marrow (MFI 2862 vs. 684, p < 0.05) (Fig. 2B).

Both M-MDSCs and G-MDSCs express CXCR4 but this was significantly higher on M-MDSCs compared to G-MDSCs in RAEB patients (p = 0.003). There was also higher CXCR4 expression on M-MDSCs derived from patients with >5% bone marrow blasts when compared to patients with <5% bone marrow blasts (p = 0.01) (Fig. 2C). There was no statistically
significant difference between healthy donor M-MDSCs and G-MDSCs in terms of CXCR4 expression.

Expression of CX3CR1 was significantly higher in M-MDSCs from patients with more than 5% bone marrow blasts (RAEB) compared to healthy donors, (median R-MFI = 10.98 vs. 5.29, \( p = 0.02 \)). CX3CR1 expression was not significantly different between patients with less than 5% bone marrow blasts also and healthy donors. The expression of CX3CR1 was significantly higher in M-MDSCs compared to G-MDSCs when we compared the CX3CR1 R-MFI for all three groups; M-MDSCs vs. G-MDSCs from RAEB patients (10.98 vs. 1.12, \( p < 0.02 \)), patients with less than 5% bone marrow blasts (8.04 vs. 1.19, \( p = 0.0005 \)), and healthy donors, (5.29 vs. 1.19, \( p = 0.003 \)). The CX3CR1 expression by G-MDSCs was not significantly different between MDS and healthy donors (Fig. 2D).
MDSCs from MDS patients inhibit T effector cell proliferation in vitro

To evaluate suppressive effect of MDSCs, T-effectors from three healthy donors were cultured in the presence and absence of MDSCs in 1:1 ratio. M-MDSCs from patients were able to suppress T effector proliferation following 5 d culture ($p = 0.03$, Figs. 3A, B). To evaluate the function of MDSCs in autologous setting, T-effectors from MDS patients (2 INTR and 2 LR) were co-cultured with autologous M-MDSCs which have shown the same level of T effector suppression ($p < 0.05$ Fig. 3C). Presence of MDSCs, also induced Tregs proliferation (Fig. 3C). MDS-MDSCs in a 1:1 ratio culture with healthy donor T-effectors were also able to suppress pro-inflammatory cytokines secretion by T-effectors (Fig. S2). The sorted MDSCs were intracellular arginase positive (data not shown).

**Discussion**

Although the immune-dysregulation is an important feature of MDS, there are important differences between low and high risk disease in terms of immunological findings. Low risk MDS is characterized by a persistent inflammatory environment in the bone marrow and expansion of autoimmune-associated T helper 17 (Th17) cells whereas expansion of Tregs and reduced number of Th17 are the characteristics of high risk MDS. However, the role of different components of the immune system and the influence of specific microenvironment on CD4+ T cell plasticity in MDS is still emerging. To investigate the potential role of MDSCs in shaping the immune response in MDS, we assessed the presence and function of circulating MDSCs in low- and high risk MDS patients. MDSCs are known to accumulate in cancer patients and tumor bearing mice, where they can inhibit adaptive anti-neoplastic immune response. The induction of MDSCs is believed to be secondary to tumor-dependent inhibition of myeloid maturation and interferes with normal immune competent antigen presenting cells. In mouse models of human cancers MDSCs mediate the development and expansion of tumor induced Tregs and Teff cell anergy. We found high numbers of MDSCs in peripheral blood of MDS patients, which is in agreement with previous reports. Nevertheless, the number of MDSCs was even higher in high risk disease compared to both low risk disease and healthy donors. A more significant expansion of MDSCs in high risk disease suggests a role for these cells in immune-surveillance suppression and facilitating the disease progression. In our cohort of patients the number of circulating G-MDSCs was higher in high risk disease and the G-MDSC subset also showed higher intracellular level of IL-10 and TGF-β compared to M-MDSCs. Our data suggest that MDSCs are not only expanded in MDS but is also correlated with risk of disease progression. In those
patients from which we had enough cells to enumerate both MDSCs and Tregs, there was a positive correlation between the number of MDSCs and Tregs in MDS. It may suggest a role for MDSCs in the in-vivo expansion of Tregs in MDS and subsequent disease progression. However, this correlation needs to be confirmed in a larger cohort of patients. While the accumulation and activation of MDSCs inside the myelodysplastic bone marrow is influenced by local factors, like S100A9,4 little is known of how these cells are recruited from the periphery. We therefore evaluated G-MDSC and M-MDSC bone marrow homing chemokine receptor expression. The CXCR4-CXCL12 axis has been shown to be involved in recruitment of MDSC to tumor microenvironment.13 Recent reports describe Fractalkine receptor CX3CR1 is also involved in bone marrow metastasis.22,27,28 Immature myeloid cells committed to the monocytic lineage express CX3CR1.29 Our data shows that MDSCs from MDS patients express both CXCR4 and CX3CR1, which facilitate migration of MDSCs to the bone marrow. Although the M-MDSCs are more frequent in the peripheral blood, they express higher levels of CX3CR1 and CXCR4 and are prone to migration toward bone marrow. Additionally, in INT + HR + VHR disease CX3CR1 was higher expressed on M-MDSCs derived from peripheral blood compared to M-MDSCs derived from bone marrow, whereas there was no significant difference in VLR + LR disease.

Figure 3. Suppression assay. (A) Healthy donor Teffector (Teff) proliferation suppresses in 1:1 co-culture with M-MDSCs from MDS patient. CFSE stained CD4+ Teff were stimulated for 5 d with anti-CD3 and anti CD28 antibodies either alone or in 1:1 co-culture with M-MDSCs. The figure is representative of one of the three suppression assays MDSs (HLA-DR-, CD14+) were isolated from fresh patient PBMCs. (B) M-MDSCs were able to significantly reduce the proliferation of allogeneic healthy donor T-effectors when co-cultured in 1:1 ratio with M-MDSCs from MDS patients. This has been repeated three times and there was a statistically difference between two conditions (p = 0.03). (C) M-MDSCs from patients were able to significantly reduce autologous T effectors proliferation (CFSE stained) p<0.05. Tregs (VPD stained) seemed to proliferate more in co-culture with MDSCs. (There was also a significant difference between unstimulated and stimulated T effectors, p < 0.05).
In summary, our data show that the expansion of MDSCs in MDS correlates with increased risk of disease progression toward AML and also positively correlates with Treg numbers in high risk MDS. The MDSCs from MDS were functional and have been able to suppress T effector function and may also induce Tregs proliferation.

The expression of homing receptors also directs these cells toward the bone marrow where they inhibit immune-surveillance against dysplastic clone(s). Inhibition of MDSCs effects (i.e. by MDSC depletion or induction of differentiation) might reverse the immunosuppressive environment and re-establish immune-surveillance in MDS.

Materials and Methods

Patients and samples

MDSCs and Treg numbers were assessed in peripheral blood and bone marrow from consecutive MDS patients seen at the outpatient clinic in the period August 2012 to September 2013 (Table 1). In total, peripheral blood from 42 pre-treatment MDS patients (median age 69.5 y) was analyzed. Twenty-three patients also had bone marrow samples available for analysis, in those cases peripheral blood and bone marrow samples were taken the same day. Twenty-seven patients had MDS without excess of bone marrow blasts; RC (N = 1), RARS (N = 5) or RCMD (N = 17), MDS-U (N = 4) and 15 had MDS with excess of blasts (RAEB-1 and RAEB-2). Thirty patients were categorized as low or very low risk disease on IPSS-R scoring system and 12 patients had intermediate or high risk disease. Peripheral blood samples from 12 healthy age matched donors, 6 females, and 6 males, median age 63.5 y (range 41–83 y) were used as controls. For three of the healthy donors, we were able to obtain bone marrow samples as well as peripheral blood. The study was approved by the local ethical committee and all samples taken after written consent.

Flow cytometry

Cell surface and intracellular staining was performed following red cell-lysis (Pharm-Lyse Lysing buffer, Becton Dickinson and Company BD Biosciences Pharmingen, San Jose, CA, USA) using the following markers: For surface staining: Live/dead eFluor780, CD33 eFluor450, Lineage markers (all APC-eFluor780; CD3, CD16, CD19, CD20, CD34, CD56, HLA-DR), all from eBioscience (San Diego, CA, USA). CD15 Pacific Orange, (Invitrogen, Carlsbad, CA, USA), CD11b PerCP/Cy5.5 BioLegend, CD66b FITC Biolegend, CD14 APC BD Biosciences, for chemokine receptor staining: CXCR3 FITC, CX3CR1 PE, CXCR4 APC, all from eBioscience. Intracellular staining was performed for IL-10 APC, TGF-β PE, Arginase 1 CFS, all from RnDSystems (Minneapolis, MN, USA), lymphocytes served as internal negative controls for each sample. The relative MFI was calculated based on the auto-fluorescence levels of each patient’s unstained cells. After permeabilization and fixation (Flow Cytometry Permeabilization/Wash Buffer I, RnDSystems) as described in30,31 flow cytometry was performed by FACSCantoII (Beckton Dickinson) and data were analyzed using FlowJo software, TreeStar, Inc., Ashland, OR, USA). MDSCs were defined as Lin−/CD33+/CD11b+, subdivided into CD15+ granulocytic MDSCs (G-MDSCs) or CD14+ monocytic MDSCs, (M-MDSCs). We have used FSC-SSC characteristics to exclude any eosinophil contamination as described before (Fig. 4A).32 As cryopreservation changes the G-MDSC numbers and function,33 all MDSC analyses were carried out on fresh peripheral blood and bone marrow samples. Tregs, T helpers and T cell subset staining were performed as per previously published protocols.34 Gating strategy for MDSCs is shown in Figure 4B as described before.33

Suppression assay

T effector cells, Tregs and HLA-DR-/CD14+ MDSC were isolated from Ficoll-separated PBMC by stepwise magnetic separation (MACS columns, Miltenyi Biotec Inc., Auburn, CA, USA). T effector cells were isolated by CD4+ negative depletion (Miltenyi Biotec Inc.) and were together with Tregs separated by CD4+CD25+ Regulatory T Cell Isolation Kit, also from Miltenyi Biotec Inc.. For MDSCs, PMBCs were first incubated with Anti-HLA-DR Micro Beads and the HLA-DR negative
population was further incubated with Anti-CD14 Micro Beads, both from Miltenyi Biotec Inc.. To evaluate the suppressive function of MDSCs, CFSE stained CD3\(^+\)CD4\(^+\)CD25\(^-\) (T-effectors) from MDS patients were stimulated by anti-human CD3 (OKT1, eBioscience) and anti-human CD28 (Functional grade purified, eBioscience) and cultured for 5 d under the following conditions: T-effectors alone, T-effectors, and Tregs (CD3\(^+\)/CD4\(^+\)/CD25\(^+\)) in 2:1 ratio; T-effectors and Tregs and MDSCs in 2:1:1 ratio. To evaluate Tregs proliferation, the CD25\(^+\) cells were stained with Violet proliferation dye (VPD).

### Table 1. Patients included in the study, with patient and disease characteristics and treatment received

| PT. | Gender | Age \(^1\) | Diagnosis \(^2\) | WHO class \(^3\) | Cytopenia(s) \(^4\) | Cytogenetics \(^5\) | Therapy \(^6\) |
|-----|--------|-------------|-----------------|-----------------|-------------------|-----------------|-----------------|
| 1   | M      | 81          | 2012            | RCMD            | A, T              | 46 XY           | BS, E           |
| 2   | M      | 78          | 2008            | RCMD            | A, T              | 46 XY           | BS              |
| 3   | M      | 82          | 2011            | RARS            | A                 | 46 XY           | BS, E           |
| 4   | F      | 87          | 1990            | RCMD            | A, T              | NA              | BS              |
| 5   | M      | 89          | 2007            | RCMD            | A, T              | 45 XY-11        | BS              |
| 6   | F      | 75          | 2006            | RARS            | A                 | NA              | BS, E           |
| 7   | M      | 56          | 2012            | RCMD            | N, T              | 47 XY +1        | BS              |
| 8   | M      | 76          | 2000            | RARS            | A, T              | 46 XY           | BS              |
| 9   | M      | 69          | 2007            | RARS            | A                 | 46 XY           | BS, E, G        |
| 10  | F      | 86          | 2012            | RCMD            | A                 | 46 XX           | BS, E           |
| 11  | M      | 89          | 2012            | RCMD/CMML-1     | A, T              | 46 XY           | BS              |
| 12  | M      | 74          | 2012            | RC               | N                 | 46 XY           | BS              |
| 13  | M      | 60          | 2011            | RCMD            | A                 | 46 XY           | BS, E           |
| 14  | F      | 70          | 2011            | RARS            | A                 | 46 XX           | BS              |
| 15  | M      | 91          | 2012            | RCMD            | A, N, T           | 46 XY           | BS, E           |
| 16  | F      | 78          | 2012            | RCMD            | A, N, T           | 46 XX           | BS              |
| 17  | M      | 94          | 2012            | RCMD            | A                 | NA              | BS, E           |
| 18  | M      | 82          | 2012            | RAEB-1          | A                 | 46 XY           | BS              |
| 19  | M      | 83          | 2010            | RAEB-1          | A                 | 46 XY           | BS              |
| 20  | F      | 49          | 2013            | RCMD            | T                 | 47 XX +6        | BS              |
| 21  | F      | 66          | 2013            | MDS-U           | A, T              | 46 XX del(13)(q12q22) | BS       |
| 22  | F      | 60          | 2013            | RCMD            | T                 | 47 XX +21       | BS              |
| 23  | M      | 68          | 2013            | RCMD            | T                 | 46 XY           | BS              |
| 24  | M      | 25          | 2013            | MDS-U           | T                 | 46 XY           | BS              |
| 25  | F      | 65          | 2014            | RAEB            | A, N              | NA              | BS              |
| 26  | F      | 49          | 2014            | RCMD            | A                 | 46 XX           | C, P            |
| 27  | M      | 49          | 2014            | MDS-U           | N, T              | 46 XY           | BS              |
| 28  | M      | 43          | 2014            | MDS-U           | N                 | 46 XX           | BS              |
| 29  | F      | 47          | 2014            | RCMD            | A                 | 46 XX           | BS              |
| 30  | F      | 64          | 2014            | RCMD            | A, N, T           | 46 XX           | BS              |

\(^1\)Age at sampling.
\(^2\)Year diagnosed with MDS.
\(^3\)RARS = Refractory anemia with ringed sideroblasts, RCMD = refractory cytopenia with multilineage dysplasia, RAEB = Refractory anemia with excess blasts, MDS-U = MDS unclassified, CMML = Chronic myelomonocytic leukemia.
\(^4\)A = anemia, N = neutropenia, T = thrombocytopenia.
\(^5\)NA = Not acquired.
\(^6\)BS = best supportive care (including transfusions), E = Epo, G = G-CSF, H = Hydroxycarbamide, A = Azacitidine, C = Ciclosporin, P = Prednisolone.

www.tandfonline.com e1062208-7 OncoImmunology
Disclosure of Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

The authors would like to thank Dr. Geir Hallan, Department for Orthopaedic surgery, Haukeland University Hospital, for healthy donor bone marrow sampling and to Professor Einar Kristoffersen at Gades Institute, University of Bergen, for helpful advice.

Funding

This work was supported by The Western Norway Regional Health Authority (Helse-Vest), the Norwegian Cancer Society, Leukemia and Lymphoma Research (LLR), UK and King’s College London.

Supplemental Material

Supplemental data for this article can be accessed on the publisher’s website.

1. Sumida K, Wakizaka D, Narita Y, Masuko K, Terada S, Watanabe K, Satoh T, Kitamura H, Nishimura T. Anti-IL-6 receptor mAb eliminates myeloid-derived suppressor cells and inhibits tumor growth by enhancing T-cell responses. Eur J Immunol 2012; 42:2060-72; PMID:22586368; http://dx.doi.org/10.1002/eji.201142335

2. Kowanesz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, Yu L, Ross J, Korisai C, Cao T et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G(+) Ly6C(-) granulocytes. Proc Natl Acad Sci U S A 2010; 107:21248-55; PMID:21081700; http://dx.doi.org/10.1073/pnas.1015855107

3. Pekarek LA, Statt BA, Tledelano AY, Schreiber H. Inhibition of tumor growth by elimination of granulocytic J Exp Med 1995; 181:435-40; PMID:7807024; http://dx.doi.org/10.1088/j Em.181.1.435

4. Obermaier N, Muthuswamy R, Odutini K, Edwards RP, Kalinski P. PGE(2)-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDS in ovarian cancer environment. Cancer Res 2011; 71:7463-70; PMID:22023564; http://dx.doi.org/10.1158/0008-5472.CAN-11-2449

5. Steinberg M, Sibora M, Plintaar A. A chemokine receptor 4 antagonist for mobilization of hematopoietic stem cells for transplantation after high-dose chemotherapy for non-Hodgkin’s lymphoma or multiple myeloma. Clin Ther 2010; 32:821-43; PMID:20684593; http://dx.doi.org/10.1016/j.clt.2010.05.007

6. Aggarwal S, de la Veodosee A, Alhan C, Olsen-koppele GJ, Westers TM, Bonkes HJ. Role of immune responses in the pathogenesis of low-risk MDS and high-risk MDS: implications for immunotherapy. Br J Haematol 2011; 153:568-81; PMID:21488861; http://dx.doi.org/10.1111/j.1365-2141.2011.08683.x

7. Matsuda M, Morita Y, Hanamoto H, Tatsumi Y, Maeda Y, Kanamura A. CD34+ progenitors from MDS patients are unresponsive to SDF-1, despite high levels of SDF-1 in bone marrow plasma. Leukemia 2004; 18:1038-40; PMID:14762446; http://dx.doi.org/10.1038/leu.2403031

8. Sukkar MB, Isa R, Xie S, Oltmanns U, Newton R, Chung KF. Fractalkine/CX3CL1 production by human airway smooth muscle cells: induction by IFN-gamma and TNF-alpha and regulation by TGF-B and corticosteroids. Am J Physiol Lung Cell Mol Physiol 2004; 287:L1230-40; PMID:15521787; http://dx.doi.org/10.1152/ajplung.00014.2004

9. Navas T, Zhou L, Esch M, Haghnavazi E, Nguyen AN, Mo Y, Pahanish P, Mohindru M, Cao T, Higgins LS et al. Inhibition of p38alpha MAPK disrupts the pathologic loop of proinflammatory factor production in the myelodysplastic syndrome bone marrow microenvironment. Leuk Lymphoma 2008; 49:1963-75; PMID:18949619; http://dx.doi.org/10.1080/10421610802322919

10. biao L, Nguyen AN, Sohal D, Ying Ma J, Pahanish P, Gundubolu K, Hayman J, Chubak A, Mo Y, Bhat BD et al. Inhibition of the TGF-B receptor I kinase promotes hematopoesis in MDS. Blood 2008; 112:3434-43; PMID:18477288; http://dx.doi.org/10.1182/blood-2008-02-139824

11. Cohen PA, Ko JS, Strokos WJ, Spencer CD, Brady JM, Gorman JE, McCarty DB, Zorzo-Manrique S, Dominguez AL, Pathanguy LB et al. Myeloid-derived suppressor cells adhere to physiologic STAT3- vs STAT5-dependent hematopoietic programming, establishing diverse tumor-mediated mechanisms of immunologic escape. Mol Immunol 2010; 121:680-710; PMID:20307741; http://dx.doi.org/10.3109/0882039.2012.703745

12. Arai A, Webb JL, Bleed C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J Exp Med 1997; 185:111-20; PMID:8996247; http://dx.doi.org/10.1088/jem.185.1.111

13. Jamieson-Gladney WL, Zhang Y, Fong AM, Marucci O, Fatatis A. The chemokine receptor CX3C1R1 is directly involved in the arrest of breast cancer cells to the skeleton. Breast Cancer Res 2011; 13:R91; PMID:21933977; 10.1186/bcr2916

14. Kordasi NY, Afzali B, Lim Z, Ingram W, Hayden J, Barber L, Matthews K, Chellah R, Lombardi MR, Wiernik A, Spellman S, Haagenson MD, Lenvik E, Fatatis A. CX3CR1 is expressed by prostate epithelial

References

1. Kordasi NY, Afzali B, Lim Z, Ingram W, Hayden J, Barber L, Matthews K, Chellah R, Lombardi MR, Wiernik A, Spellman S, Haagenson MD, Lenvik E, Fatatis A. CX3CR1 is expressed by prostate epithelial...
cells and androgens regulate the levels of CX3CL1/fractalkine in the bone marrow: potential role in prostate cancer bone tropism. Cancer Res 2008; 68:1715-22; PMID:18339851; http://dx.doi.org/10.1158/0008-5472.CAN-07-1315
28. Shulby SA, Dolloff NG, Stearns ME, Meucci O, Fattatis A. CX3CR1-fractalkine expression regulates cellular mechanisms involved in adhesion, migration, and survival of human prostate cancer cells. Cancer Res 2004; 64:4693-8; PMID:15256432; http://dx.doi.org/10.1158/0008-5472.CAN-03-3437
29. Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, Littman DR, Cumano A, Geissmann F. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science 2006; 311:83-7; PMID:16322423; http://dx.doi.org/10.1126/science.1117729
30. Garba ML, Frelinger JA. Intracellular cytokine staining for TGF-β. J Immunol Methods 2001; 258:193-8; PMID:11684136; http://dx.doi.org/10.1016/S0022-1759(01)00491-4
31. Kreft B, Singer GG, Diaz-Gallo C, Kelley VR. Detection of intracellular interleukin-10 by flow cytometry. J Immunol Methods 1992; 156:125-8; PMID:1431159; http://dx.doi.org/10.1016/0022-1759(92)90018-O
32. Sabroe I, Jones EC, Usher LR, Whyte MK, Dower SK. Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. J Immunol 2002; 168:4701-10; PMID:11971020; http://dx.doi.org/10.4049/jimmunol.168.9.4701
33. Kotsakis A, Harasymczuk M, Schilling B, Georgoulas V, Ageris A, Whiteside TL. Myeloid-derived suppressor cell measurements in fresh and cryopreserved blood samples. J Immunol Methods 2012; 381:14-22; PMID:22522114; http://dx.doi.org/10.1016/j.jim.2012.04.004
34. Kordasti S, Marsh J, Al-Khan S, Jiang J, Smith A, Mohamedali A, Abellan PP, Veen C, Costantini B, Kulasekaranaj AG et al. Functional characterization of CD4+ T cells in aplastic anemia. Blood 2012; 119:2033-43; PMID:22138514; http://dx.doi.org/10.1182/blood-2011-08-368308