Leukemia Research 37 (2013) 541–546

Contents lists available at SciVerse ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

IL10 inversely correlates with the percentage of CD8$^+$ cells in MDS patients

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ARTICLE INFO

Article history:
Received 18 October 2012
Received in revised form 4 January 2013
Accepted 25 January 2013
Available online 28 February 2013

Keywords:
Myelodysplastic Syndrome
IL10
CD8$^+$ cells
Immune System

ABSTRACT

The role of the immune system in myelodysplastic syndrome (MDS) progression has been widely accepted, although mechanisms underlying this immune dysfunction are not clear. CD4$^+$ and CD8$^+$ lymphocyte profiles in the peripheral blood of MDS patients were evaluated and correlated with clinical characteristics, the expression of *FOXP3* and the anti-inflammatory cytokines *IL10*, *TGFβ1* and *CTLA4*. *IL10* expression inversely correlated with the percentage of CD8$^+$ cells and was higher in high-risk MDS. Our findings provide further evidence for the role of T cell-mediated IL10 production in MDS and strengthen the idea of distinct cytokine profiles in low and high-risk MDS.

1. Introduction

Myelodysplastic syndrome (MDS) is characterized by an increased programmed cell death of bone marrow (BM) cells, both clonal and nonclonal precursors, which contributes to ineffective hematopoiesis and peripheral cytopenias [1–5]. Although the complex pathogenesis of MDS remains poorly defined, several studies indicate a role for the immune system in the progression of early MDS to the advanced stage [6,7]. Low-risk MDS is characterized by excessive apoptosis in the BM and by an autoimmune disease-like profile; whereas advanced MDS is distinguished by immune evasion, lower apoptosis and secondary DNA damage, facilitating the progress into acute leukemia [7,8]. Immunosuppressive and immunomodulatory therapeutics have presented favorable results, such as abrogation of transfusion dependence for a subset of the patients [8–12].

Although regulatory (Tregs) and cytotoxic T cells are reported to be modulated during the course of MDS [13], the exact mechanism by which these cells contribute to MDS progression is not yet clear. Low numbers of Tregs in low-risk MDS are associated with T cell cytotoxicity of BM precursor cells, whereas higher frequencies of Tregs in high-risk MDS result in a suppression of immune response [8,14,15].

In an attempt to better understand the role of the immune system in MDS, we evaluated CD4$^+$ and CD8$^+$ lymphocyte profiles in the peripheral blood of MDS patients. These data were correlated with clinical characteristics, the expression of *FOXP3* and the anti-inflammatory cytokines, *IL10, TGFβ1 and CTLA4*.

2. Materials and methods

2.1. Patients and healthy donors

Peripheral blood samples, collected from 49 patients with MDS and 29 unrelated, random, and healthy individuals (median age = 39, range, 28–60), were analyzed. All patients that attended the clinic between 2010 and 2011, with a confirmed diagnosis of MDS and untreated at the time of the study were included. All healthy controls and patients provided informed written consent and the study was approved by the ethics committee of the University of Campinas. Patients' characteristics are described in Table 1.

2.2. Peripheral blood analyses

Hematological values were determined with a CELL-DYN Sapphire automated hematology analyzer (Abbott Diagnostics, Illinois, USA). Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque gradient centrifugation (Sigma, St Louis, MO). PBMC were stained with the conjugated monoclonal antibodies; allophycocyanin (APC) anti-CD3, fluorescein isothiocyanate (FITC) anti-CD8, and phycoerythrin (PE) anti-CD4. An FSC/SSC gate was created around the viable lymphocyte population for further analysis of CD3$^+$ cells, CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ subsets. Data acquisition was performed using a FACScalibur Flow Cytometer

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http://dx.doi.org/10.1016/j.leukres.2013.01.019

Table 1
Clinical characteristics of patients.

Characteristics	Value
Age *y*, median (range)	67 (27–89)
Sex, *n* (%)	
Male/female	24 (49)/25 (51)
WHO classification, *n* (%)	
RCUD	09 (19)
RCMD	23 (47)
RARS	8 (16)
RAEB1	7 (14)
RAEB2	2 (4)
Risk stratification by WHO[a], *n* (%)	
Low-risk	40 (82)
High-risk	9 (18)
Cytogenetic risk group, *n* (%)	
Good	41 (84)
Intermediate	3 (6)
Poor	2 (4)
No growth	3 (6)
Peripheral blood counts, median (range)	
Hemoglobin, (g/dL)	10.5 (5.5–15.6)
White blood cell count, ($\times 10^9$/L)	3.58 (0.86–9.8)
Neutrophils count, ($\times 10^9$/L)	1.63 (0.16–6.51)
Platelet count, ($\times 10^9$/L)	158 (0.7–648)
Number of cytopenia, (%)	
0	6 (12)
1	20 (41)
2	20 (41)
3	3 (6)
Bone marrow blasts, %, median (range)	1.5 (0–14)

RCUD indicates refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; RARS, refractory anemia with ring sideroblasts; RAEB-1/2, refractory anemia with excess blasts-1/2.

[a] Low-risk includes RCUD, RARS, RCMD. High-risk includes RAEB-1/2.

(Becton Dickinson, Franklin Lakes, NJ) and analyses were carried out using CellQuest and BD FACSDiva software (Becton Dickinson, Franklin Lakes, NJ). The $CD3^+$ T cells from PBMC were sorted using anti-CD3 monoclonal antibody and MACS® Magnetic Cell sorting technique (Miltenyi Biotec, Bergisch Gladbach, Germany).

2.3. Quantitative polymerase chain reaction

Sorted $CD3^+$ cells were submitted to RNA extraction. Quantitative PCR (q-PCR) was performed in an ABI 7500 Sequence Detector System (Applied Biosystems, Foster City, CA) with specific primers for *FOXP3, IL10, TGFβ1, CTLA4*, and *HPRT* (sequences upon request). The relative gene expression was calculated using the equation, $2^{-\Delta\Delta CT}$ [16].

2.4. Statistical analysis

The age-adjusted multivariate linear regression analysis was used in order to study the influence of both age and disease on all parameters evaluated in peripheral blood [15,17]. The model included age, group (patients *vs* controls) and an interaction term (age *vs* disease status) as independent variables. The interaction term between age and disease status was dropped from the final model when not statistically significant ($P > 0.05$). Two-tailed Spearman's correlation coefficient, univariate and stepwise multivariate models were also used. Numeric variables without normal distribution were transformed into ranks for analysis. A two-sided $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Distinct profiles of peripheral blood lymphocytes exist in MDS

We observed a significant decrease in lymphocyte count in the MDS group compared to the control group after adjusting for age ($P = 0.002$, Fig. 1A). This statistical difference remained after we classified the patients into subgroups, according to WHO, but the decreased lymphocyte count was more pronounced in the high-risk MDS ($P < 0.001$, Fig. 1B). Lymphopenia ($<1.1 \times 10^9$ cells/L) was found in 13 (26.5%) of 49 MDS patients.

Age-adjusted percentages of $CD3^+$ cells were significantly higher in the MDS group ($P = 0.004$, Fig. 2A). Analyses of the $CD3^+$ cell subsets presented no statistical differences for $CD3^+CD4^+$ cells (Fig. 2C), but showed a strong trend toward an increased percentage of $CD3^+CD8^+$ cells in the MDS individuals ($P = 0.05$, Fig. 2E). Comparisons between the subgroups of the disease showed higher $CD3^+$ frequencies in the high-risk, compared to the low-risk MDS ($P = 0.02$, Fig. 2B), followed by higher $CD3^+CD4^+$ frequencies in the high-risk compared to the control group ($P = 0.02$, Fig. 2D). The frequency of $CD3^+CD8^+$ cells was significantly higher in the low-risk MDS, when compared with the control group ($P = 0.04$, Fig. 2F).

There was no statistical difference between the MDS and the control groups with regard to the CD4:CD8 ratios (Fig. 2G); however comparison among MDS patients revealed a significantly higher CD4:CD8 ratio in the high-risk, compared to the low-risk groups ($P = 0.03$, Fig. 2H).

We also correlated $CD3^+CD4^+$ and $CD3^+CD8^+$ cell frequencies with clinical data (age, sex, hemoglobin, leukocyte, granulocyte, platelet, number of cytopenias, percentage of blasts in BM, and karyotype risk group). Univariate analysis demonstrated that advanced age correlated with a decreased percentage of $CD3^+CD8^+$ cells (Beta = −0.28; $P = 0.037$; $R^2 = 0.11$), which was confirmed by multivariate analysis (Beta = −0.30; $P = 0.024$; $R^2 = 0.14$). There was no significant correlation between $CD3^+CD4^+$ cell frequency and the clinical parameters studied.

3.2. IL10 inversely correlates with the percentage of $CD8^+$ cells and presents higher expression in high-risk MDS

To better understand the regulation of the anti-inflammatory cytokines *IL10, TGFβ1, and CTLA4*, as well as *FOXP3*, in MDS, the

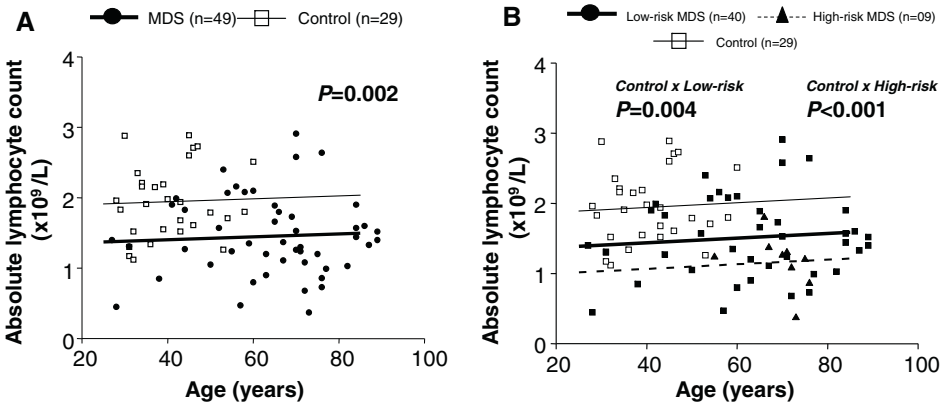

Fig. 1. Peripheral blood absolute lymphocyte count in MDS and control groups. (A–B) Multivariate regression analysis was performed with lymphocyte count, as the dependent variable, and age and disease status as independent variables. The *P* value and the number of individuals are shown in the figure. Low and high-risk MDS, according to WHO classification.

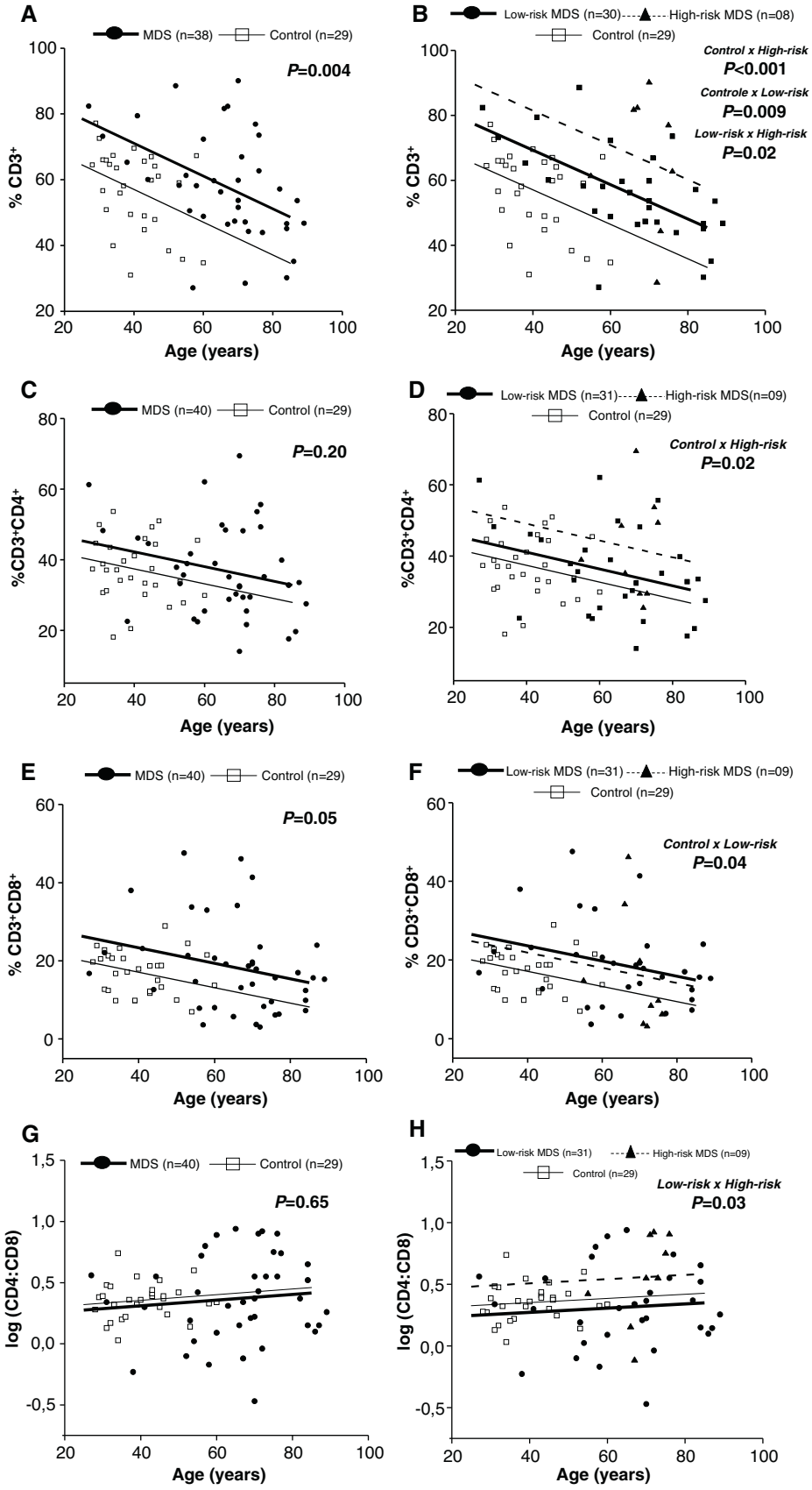

Fig. 2. $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cell profiles in MDS and control groups. Multivariate regression analysis was performed with $\%CD3^+$ (A–B), $\%CD3^+CD4^+$ (C–D), $\%CD3^+CD8^+$ (E–F), and log-transformed ratio of CD4:CD8 (G–H), as the dependent variables, and age and disease status as independent variables. The *P* value and the number of individuals are shown in the figure. Patients were subgrouped into low and high-risk MDS, according to WHO.

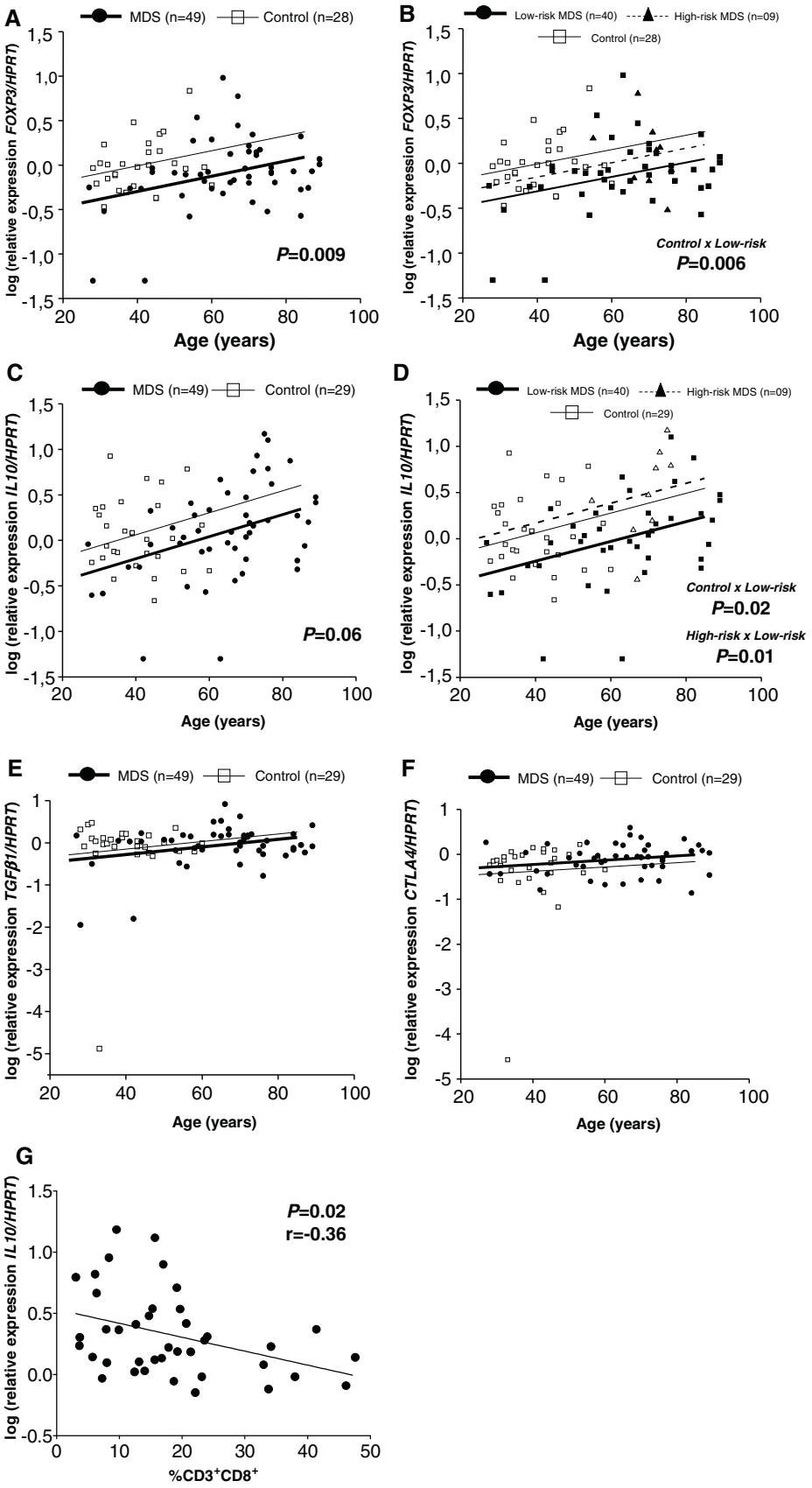

Fig. 3. *FOXP3*, *IL10*, *TGFβ1* and *CTLA4* expressions in the peripheral blood $CD3^+$ cells of MDS and control groups. Multivariate regression analysis was performed with the log-transformed relative expression of *FOXP3/HPRT* (A-B), *IL10/HPRT* (C-D), *TGFβ1/HPRT (E), and CTLA4/HPRT (F)* as dependent variables and age and disease status as independent variables. Patients were subgrouped into low and high-risk MDS, according to WHO. (G) Relationship between the log-transformed relative expression of *IL10/HPRT* and the frequency of $CD3^+CD8^+$ cells in the peripheral blood of MDS patients. Spearman's correlation. The *P* value and the number of individuals are shown in the Figure.

transcript expressions of these genes were analyzed in peripheral CD3$^+$ cells. We observed a decrease in *FOXP3* expression in the MDS group, when compared with the control group after adjusting for age ($P = 0.009$, Fig. 3A). Similar statistical differences remained when low-risk MDS was compared with the control group ($P = 0.006$, Fig. 3B). *IL10, TGFβ1*, and *CTLA4* correlated positively with *FOXP3* expression, according to Spearman's correlation ($P = 0.008$, $r = 0.37$; $P = 0.04$, $r = 0.28$; $P = 0.01$, $r = 0.35$, respectively). Secondly, there was a trend toward a lower *IL10* expression in the MDS group, when compared with the control group ($P = 0.06$, Fig. 3C), probably due to the lower *IL10* expression observed in the low-risk MDS, when compared to the control group ($P = 0.02$, Fig. 3D). Additionally, higher levels of *IL10* transcripts in the high-risk, compared to the low-risk MDS, were observed ($P = 0.01$, Fig. 3D). No significant differences in *TGFβ1* and *CTLA4* expressions were observed (Fig. 3E-F). *IL10* transcripts inversely correlated with CD3$^+$CD8$^+$ frequency in MDS patients (Spearman $r = -0.36$; $P = 0.02$, Fig. 3G); however, there were no correlations between the cytokines analyzed and CD3$^+$CD4$^+$ frequency.

4. Discussion

There is a clear involvement of multiple myeloid cell lineages in the MDS clone, which results in the loss of the capacity of differentiation and apoptosis in the bone marrow, with consequent peripheral pancytopenia in patients [18,19]. Conversely, several studies have shown that, in most cases of MDS, the lymphocytes are not involved in the malignant clone [20–22]. In our study, there was a significant decrease in the absolute lymphocyte counts in the peripheral blood of MDS patients, which is in accordance with the literature [21,23,24] and supports the immunological abnormalities that have been extensively described in MDS patients. Lymphopenia in MDS has been suggested to occur due to a decrease in the T cell numbers, as a consequence of T cell apoptosis in the peripheral blood of MDS patients [23,25]; as well as a consequence of CD19$^+$ cell apoptosis in both the peripheral blood and bone marrow of MDS patients [21,26]. Additionally, a decreased NKT cell number, already described in MDS patients [27], could contribute to the lower absolute lymphocyte count. Further studies addressing the absolute values of each cell type in the peripheral blood of MDS patients could clarify which specific cells are involved in the lower absolute lymphocyte counts. Although our findings show that only 26.5% of patients with lymphopenia, the most prominent decrease in lymphocyte counts was in the high-risk group, which is in agreement with the preview reports [21,28].

Data presented herein show that the increase in CD3$^+$ cell percentage in MDS may be a reflection of CD8$^+$ frequency in the low-risk group and CD4$^+$ frequency in the high-risk group. The increased CD8$^+$ frequency in the low-risk MDS is in agreement with other studies [29–35] and supports the contribution of CD8$^+$ cells to the apoptosis of hematopoietic progenitors, since the early stages of this disease are characterized by an increased apoptotic activity [7].

In contrast to previous reports demonstrating no significant differences in the CD4$^+$ cell frequency between patients and controls [36,37], we showed a higher CD4$^+$ cell frequency in high-risk MDS, with a consequently increased CD4:CD8 ratio in this subgroup. In fact, studies of T cell subsets in MDS have been contradictory; a decreased CD4:CD8 ratio in MDS patients has been reported [15,38], while other studies have shown an increased CD4:CD8 ratio in intermediate and high-risk MDS [39]. It has been shown that the inversion of the CD4:CD8 ratio is associated with the response to immunosuppressive therapy (IST) and is inversely correlated with the proliferative T-cell index before IST in these patients [15]. Taken together, our results suggest the contribution of T CD4$^+$ cells to the pathophysiology of the disease.

The majority of CD4$^+$ Treg cells present specific FOXP3 expression, a transcription factor, which is important for the development and function of these cells [40]; however, a population of CD8$^+$FOXP3$^+$ T cells has been described in several autoimmune diseases, after allergen exposure and allogenic transplantation [41–46]. We found that the lower expression of FOXP3 transcripts in the peripheral CD3$^+$ cells of MDS patients was clearly due to the lower expression of this gene in the low-risk group. The only study that reports on CD8$^+$ Treg cells in MDS, related no difference in the number of CD8$^+$ Treg cells between MDS groups, IPSS or disease progression [14]. Although data regarding CD4$^+$ Treg frequency in the low-risk MDS patients are uncertain, mainly due to the different flow cytometry strategies used [47], our data for FOXP3 expression support studies that report that the number of CD4$^+$ Tregs is lower in low-risk MDS [7,14].

Although IL10 and TGFβ1 are secreted by many cell types, the production of IL10 and TGFβ1, as well as the expression of CTLA4, are indications of the activation of Treg cells [40]. Our results in CD3$^+$ cells showed a significant positive correlation between the expression of these regulatory molecules and *FOXP3* expression, indicating that these transcripts are derived from Treg cells. *IL10* and *FOXP3* expressions were lower in the low-risk group, compared to the control group, corroborating the hypothesis of down-regulated Tregs in low-risk MDS [7].

A significant increase in *IL10* expression was observed in high-risk, compared to low-risk MDS, which is in agreement with the measurement of IL10 concentrations in serum, described by Kordasti et al. [48]. Interestingly, we observed an inverse correlation between CD8$^+$ cell frequency and *IL10* expression, supporting data describing the recruitment of CD8$^+$ cells in an inverse relationship with the levels of Tregs in the bone marrow of MDS patients [49]. IL10 is thought to contribute to the immune suppressive milieu, by inhibition of antigen presentation, cytokine expression and T helper cell functions [50,51], all features of the immune evasion that is characteristic of high-risk MDS [7]. We postulate that IL10, secreted by Tregs, may have a role in the prevention of MDS clone elimination, with a consequent role in MDS progression.

In conclusion, our findings provide further evidence for Treg deregulation in low-risk MDS; and most importantly, add new insight into the role of T cell-mediated IL10 production in MDS and strengthen the idea of distinct cytokine profiles in low and high-risk MDS.

Funding

This work received financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). The Hematology and Hemotherapy Center – UNICAMP forms part of the National Institute of Blood, Brazil (INCT de Sangue–CNPq/MCT).

Authors' Contributions

MRL: carried out all experiments and participated in the writing of the manuscript. FT, PMC and SCOG: responsible for collection of patient samples and clinical data, and participated in the edition of the manuscript. JKNP, JAMN: helped with the experiments, analysis, and edition of the manuscript. HCM: carried out all the statistical analysis and contributed to manuscript writing. STOS: contributed to the study design, data analyses, and manuscript writing. PF was the principal investigator and takes primary responsibility for the paper.

Conflict of interest

Authors have no conflicts of interest.

Acknowledgements

The authors would like to thank Nicola Conran and Raquel Susana Foglio for English review. The authors would also like to thank Tereza Salles for her invaluable technical assistance.

References

[1] Deeg HJ, Beckham C, Loken MR, Bryant E, Lesnikova M, Shulman HM, Gooley T. Negative regulators of hemopoiesis and stroma function in patients with myelodysplastic syndrome. Leuk Lymphoma 2000;37:405–14.
[2] Mufti G, List AF, Gore SD, Ho AY. Myelodysplastic syndrome. Hematology Am Soc Hematol Educ Program 2003:176–99.
[3] Parker JE, Mufti GJ. Ineffective hematopoiesis and apoptosis in myelodysplastic syndromes. Br J Haematol 1998;101:220–30.
[4] Parker JE, Mufti GJ. Excessive apoptosis in low risk myelodysplastic syndromes (MDS). Leuk Lymphoma 2000;40:1–24.
[5] Parker JE, Mufti GJ, Rasool F, Mijovic A, Devereux S, Pagliuca A. The role of apoptosis, proliferation, and the Bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. Blood 2000;96:3932–8.
[6] Barrett AJ, Sloand E. Autoimmune mechanisms in the pathophysiology of myelodysplastic syndromes and their clinical relevance. Haematologica 2009;94:449–51.
[7] Aggarwal S, van de Loosdrecht AA, Alhan C, Ossenkoppele GJ, Westers TM, Bontkes 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.
[8] Kotsianidis I, Bouchliou I, Nakou E, Spanoudakis E, Margaritis D, Christophoridou AV, Anastasiades A, Tsigalou C, Bourikas G, Karadimitris A, Tsatalas C. Kinetics, function and bone marrow trafficking of CD4+CD25+FOXP3+ regulatory T cells in myelodysplastic syndromes (MDS). Leukemia 2009;23:510–8.
[9] Ortega J, List A. Immunomodulatory drugs in the treatment of myelodysplastic syndromes. Curr Opin Oncol 2007;19:656–9.
[10] Greenberg PL, Young NS, Gattermann N. Myelodysplastic syndromes. Hematology Am Soc Hematol Educ Program 2002:136–61.
[11] Molldrem JJ, Caples M, Mavroudis D, Plante M, Young NS, Barrett AJ. Antithymocyte globulin for patients with myelodysplastic syndrome. Br J Haematol 1997;99:699–705.
[12] Killick SB, Mufti G, Cavenagh JD, Mijovic A, Peacock JL, Gordon-Smith EC, Bowen DT, Marsh JC. A pilot study of antithymocyte globulin (ATG) in the treatment of patients with 'low-risk' myelodysplasia. Br J Haematol 2003;120:679–84.
[13] Fozza C, Longinotti M. Are T-cell dysfunctions the other side of the moon in the pathogenesis of myelodysplastic syndromes? Eur J Haematol 2012;88:380–7.
[14] Kordasti SY, Ingram W, Hayden J, Darling D, Barber L, Afzali B, Lombardi G, Wlodarski MW, Maciejewski JP, Farzaneh F, Mufti GJ. CD4+CD25high Foxp3+ regulatory T cells in myelodysplastic syndrome (MDS). Blood 2007;110:847–50.
[15] Zou JX, Rollison DE, Boulware D, Chen DT, Sloand EM, Pfannes LV, Goronzy JJ, Bai F, Painter JS, Wei S, Cosgrove D, List AF, Epling-Burnette PK. Altered naive and memory CD4+ T-cell homeostasis and immunosenescence characterize younger patients with myelodysplastic syndrome. Leukemia 2009;23:1288–96.
[16] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402–8.
[17] Tabachnick BG, Fidell LS. Using Multivariate Statistics. Boston: Allyn and Bacon; 2001. p. 111–176.
[18] Yoshida Y, Stephenson J, Mufti GJ. Myelodysplastic syndromes: from morphology to molecular biology. Part I. Classification, natural history and cell biology of myelodysplasia. Int J Hematol 1993;57:87–97.
[19] Bogdanovic AD, Trpinac DP, Jankovic GM, Bumbasirevic VZ, Obradovic M, Colovic MD. Incidence and role of apoptosis in myelodysplastic syndrome: morphological and ultrastructural assessment. Leukemia 1997;11:656–9.
[20] Nilsson L, Astrand-Grundstrom I, Arvidsson I, Jacobsson B, Hellstrom-Lindberg E, Hast R, Jacobsen SE. Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level. Blood 2000;96:2012–21.
[21] Shioi Y, Tamura H, Yokose N, Satoh C, Dan K, Ogata K. Increased apoptosis of circulating T cells in myelodysplastic syndromes. Leuk Res 2007;31:1641–8.
[22] Nimer SD. MDS: a stem cell disorder – but what exactly is wrong with the primitive hematopoietic cells in this disease? Hematology Am Soc Hematol Educ Program 2008:43–51.
[23] Bynoe AG, Scott CS, Ford P, Roberts BE. Decreased T helper cells in the myelodysplastic syndromes. Br J Haematol 1983;54:97–102.
[24] Hamblin TJ. Immunological abnormalities in myelodysplastic syndromes. Semin Hematol 1996;33:150–62.
[25] Hamblin T. Immunologic abnormalities in myelodysplastic syndromes. Hematol Oncol Clin North Am 1992;6:571–86.
[26] Amin HM, Jilani I, Estey EH, Keating MJ, Dey AL, Manshouri T, Kantarjian HM, Estrov Z, Cortes JE, Thomas DA, Giles FJ, Albitar M. Increased apoptosis in bone marrow B lymphocytes but not T lymphocytes in myelodysplastic syndrome. Blood 2003;102:1866–8.
[27] Chan AC, Neeson P, Leeansyah E, Tainton K, Quach H, Prince HM, Godfrey DI, Ritchie D, Berzins SP. Testing the NKT cell hypothesis in lenalidomide-treated myelodysplastic syndrome patients. Leukemia 2010;24:592–600.
[28] Hamada K, Takahashi I, Matsuoka M, Saika T, Mizobuchi N, Yorimitsu S, Takimoto H. Apoptosis of peripheral leukocytes in patients with myelodysplastic syndromes. Rinsho Ketsueki 1998;39:1079–84.
[29] Matsutani T, Yoshioka T, Tsuruta Y, Shimamoto T, Ohyashiki JH, Suzuki R, Ohyashiki K. Determination of T-cell receptors of clonal CD8-positive T-cells in myelodysplastic syndrome with erythroid hypoplasia. Leuk Res 2003;27:305–12.
[30] Epling-Burnette PK, Painter JS, Rollison DE, Ku E, Vendron D, Widen R, Boulware D, Zou JX, Bai F, List AF. Prevalence and clinical association of clonal T-cell expansions in Myelodysplastic Syndrome. Leukemia 2007;21:659–67.
[31] Fozza C, Contini S, Galleu A, Simula MP, Virdis P, Bonfigli S, Longinotti M. Patients with myelodysplastic syndromes display several T-cell expansions, which are mostly polyclonal in the CD4(+) subset and oligoclonal in the CD8(+) subset. Exp Hematol 2009;37:947–55.
[32] Kook H, Zeng W, Guibin C, Kirby M, Young NS, Maciejewski JP. Increased cytotoxic T cells with effector phenotype in aplastic anemia and myelodysplasia. Exp Hematol 2001;29:1270–7.
[33] Sloand EM, Melenhorst JJ, Tucker ZC, Pfannes L, Brenchley JM, Yong A, Visconte V, Wu C, Gostick E, Scheinberg P, Olnes MJ, Douek DC, Price DA, Barrett AJ, Young NS. T-cell immune responses to Wilms tumor 1 protein in myelodysplasia responsive to immunosuppressive therapy. Blood 2011;117:2691–9.
[34] Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. Blood 2002;99:3129–35.
[35] Sloand EM, Mainwaring L, Fuhrer M, Ramkissoon S, Risitano AM, Keyvanafar K, Lu J, Basu A, Barrett AJ, Young NS. Preferential suppression of trisomy 8 compared with normal hematopoietic cell growth by autologous lymphocytes in patients with trisomy 8 myelodysplastic syndrome. Blood 2005;106:841–51.
[36] Chamuleau ME, Westers TM, van Dreunen L, Groenland J, Zevenbergen A, Eeltink CM, Ossenkoppele GJ, van de Loosdrecht AA. Immune mediated autologous cytotoxicity against hematopoietic precursor cells in patients with myelodysplastic syndrome. Haematologica 2009;94:496–506.
[37] Meers S, Vandenberghe P, Boogaerts M, Verhoef G, Delforge M. The clinical significance of activated lymphocytes in patients with myelodysplastic syndromes: a single centre study of 131 patients. Leuk Res 2008;32:1026–35.
[38] Symeonidis A, Kourakli A, Katevas P, Perraki M, Tiniakou M, Matsouka P, Georgoulias V, Zoumbos N. Immune function parameters at diagnosis in patients with myelodysplastic syndromes: correlation with the FAB classification and prognosis. Eur J Haematol 1991;47:277–81.
[39] Hamdi W, Ogawara H, Handa H, Tsukamoto N, Murakami H. Clinical significance of Th1/Th2 ratio in patients with myelodysplastic syndrome. Int J Lab Hematol 2009;31:630–8.
[40] Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057–61.
[41] Hahn BH, Singh RP, La Cava A, Ebling FM. Tolerogenic treatment of lupus mice with consensus peptide induces Foxp3-expressing, apoptosis-resistant, TGFbeta-secreting CD8+ T cell suppressors. J Immunol 2005;175:7728–37.
[42] Wong M, La Cava A, Singh RP, Hahn BH. Blockade of programmed death-1 in young (New Zealand black x New Zealand white)F1 mice promotes the activity of suppressive CD^{8+} T cells that protect from lupus-like disease. J Immunol 2010;185:6563–71.
[43] Frisullo G, Nociti V, Iorio R, Plantone D, Patanella AK, Tonali PA, Batocchi AP. CD8(+)Foxp3(+) T cells in peripheral blood of relapsing-remitting multiple sclerosis patients. Hum Immunol 2010;71:437–41.
[44] Tsai YG, Yang KD, Niu DM, Chien JW, Lin CY. TLR2 agonists enhance CD^{8+} $Foxp3^{+}$ regulatory T cells and suppress Th2 immune responses during allergen immunotherapy. J Immunol 2010;184:7229–37.
[45] Zhou H, Wang ZD, Zhu X, You Y, Zou P. $CD8^{+}$ $FOXP3^{+}$ T cells from renal transplant recipients in quiescence induce immunoglobulin-like transcripts-3 and -4 on dendritic cells from their respective donors. Transplant Proc 2007;39:3065–7.
[46] Lerret NM, Houlihan JL, Kheradmand T, Pothoven KL, Zhang ZJ, Luo X. Donor-specific CD8+ Foxp3+ T cells protect skin allografts and facilitate induction of conventional CD^{4+} $Foxp^{3+}$ regulatory T cells. Am J Transplant 2012;12:2335–47.
[47] Fozza C, Longu F, Contini S, Galleu A, Virdis P, Bonfigli S, Murineddu M, Gabbas A, Longinotti M. Patients with Early-Stage Myelodysplastic Syndromes Show Increased Frequency of CD4+CD25+CD127(low) Regulatory T Cells. Acta Haematol 2012;128:178–82.
[48] Kordasti SY, Afzali B, Lim Z, Ingram W, Hayden J, Barber L, Matthews K, Chelliah R, Guinn B, Lombardi G, Farzaneh F, Mufti GJ. IL-17-producing CD4(+) T cells, pro-inflammatory cytokines and apoptosis are increased in low risk myelodysplastic syndrome. Br J Haematol 2009;145:64–72.
[49] Alfinito F, Sica M, Luciano L, Pepa RD, Palladino C, Ferrara I, Giani U, Ruggiero G, Terrazzano G. Immune dysregulation and dyserythropoiesis in the myelodysplastic syndromes. Br J Haematol 2009;148:90–8.
[50] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683–765.
[51] Kang SS, Allen PM. Priming in the presence of IL-10 results in direct enhancement of CD8+ T cell primary responses and inhibition of secondary responses. J Immunol 2005;174:5382–9.