Circulating levels of MCP-1, sIL-2R, IL-15, and IL-8 predict anemia response to pomalidomide therapy in myelofibrosis

Animesh Pardanani, Kebede Begna, Christy Finke, Terra Lasho and Ayalew Tefferi

Cytokine-phenotype associations have recently been described in primary myelofibrosis and increased levels of IL-8, sIL-2R, IL-12, and IL-15 were found to be independently predictive of inferior survival. Pomalidomide therapy is effective for alleviating anemia in myelofibrosis; we examined the relationship between plasma cytokine/chemokine levels and response to treatment with pomalidomide. The study population included 32 Mayo Clinic patients (median age 66 years) who participated in two consecutive clinical trials of pomalidomide therapy for myelofibrosis-associated anemia. Ten (31%) patients achieved anemia response per International Working Group criteria. Anemia response was seen only in the presence of *JAK2*V617F ($P = 0.04$) and, in addition, predicted by lower circulating levels of MCP-1 ($P = 0.003$), IL-2R ($P = 0.008$), IL-15 (0.01), and IL-8 ($P = 0.02$). Marked splenomegaly and increased serum LDH level were associated with poor response ($P = 0.02$ and 0.03, respectively) and with each other ($P = 0.02$), but not with *JAK2*V617F. The aforementioned cytokines were not significantly associated with *JAK2*V617F but increased levels of sIL-2R ($P = 0.01$), IL-15 ($P = 0.06$), and MCP-1 ($P = 0.07$) clustered with marked splenomegaly. Current data suggest that, in the context of pomalidomide treatment, response is more likely in the presence of *JAK2*V617F and further predicted by the absence of marked splenomegaly or increased levels of proinflammatory cytokines. Am. J. Hematol. 86:343–345, 2011. © 2011 Wiley-Liss, Inc.

Introduction

The clinical manifestations of *BCR-ABL1*-negative myeloproliferative neoplasms (MPN), particularly primary (PMF), post-polycythemia vera (post-PV MF), or post-essential thrombocythemia (post-ET MF) myelofibrosis stem not just from expansion of the malignant clone but also from abnormal expression of pro-inflammatory and pro-angiogenic cytokines [1]. Current dogma holds that bone marrow collagen fibrosis and osteosclerosis in the aforementioned conditions reflect a cytokine-mediated secondary inflammatory response that is triggered by the abnormal interaction between clonal myeloid cells and polyclonal bone marrow stromal cells [2]. Various studies utilizing either tissue specimens from MPN patients or murine models that develop myelofibrosis have implicated either megakaryocytes/platelets [3–5] or monocyte/macrophage lineage cells [6,7] as the key source for abnormal cytokine synthesis.

Recent studies have indicated the clinical relevance of elevated circulating cytokines both from the standpoint of refining disease prognostication, as well as potential targets for therapeutic intervention. In a recent comprehensive cytokine profiling study, plasma concentrations of 30 cytokines/chemokines were determined in a multiplexed assay using archived samples from 127 PMF patients [8]. Concentrations of 19 cytokines/chemokines were significantly elevated relative to normal controls; specific cytokine-phenotype associations were identified including increased interleukin (IL)-8 levels and presence of constitutional symptoms or increased circulating blasts, increased IL-2R/IL-12 levels and red blood cell transfusion need, and increased IL-2R/IL-8 levels and leukocytosis. Increased concentrations of four cytokines, namely IL-8, IL-2R, IL-12, and IL-15 were independently predictive of inferior survival in multivariable analysis that included the Dynamic International Prognostic Scoring System (DIPSS)-plus model [9].

Other recent studies using novel ATP-mimetic JAK-1/2 inhibitors have demonstrated a significant down-regulation of circulating pro-inflammatory/angiogenic cytokines in murine models of myelofibrosis [10,11] as well as in myelofibrosis patients treated with some, [12] but not other inhibitors [13]. These observations lend credence to the hypothesis that the substantial palliative benefits (especially improved constitutional symptoms) with some JAK-1/2 inhibitors ensue in large part from their anti-cytokine activity, rather than any significant anti-clonal activity [12]. These drugs have relatively modest, if any, effect on disease-related parameters such as leukocytosis/thrombocytosis, bone marrow histology, or genomic (*JAK2*V617F) burden and it remains to be seen whether targeting abnormal cytokine levels with such agents has any disease-modifying value with longer-term treatment.

Pomalidomide, like lenalidomide, is a thalidomide derivative; the two immunomodulatory drugs (IMiDs) exhibit pleiotropic properties in cancer models, including anti-angiogenic, anti-proliferative, anti-inflammatory, anti-T regulatory cell, pro-T and NK cell, and pro-erythropoietic activities, although the exact cellular targets/mechanisms remain unclear [14]. Pomalidomide has demonstrated efficacy for improving anemia in myelofibrosis patients; [15–17] in one study, the anemia response was limited to patients who were *JAK2*V617F-positive [15]. Interestingly however, unlike the JAK-1/2 inhibitors, pomalidomide shows little, if any, activity at improving either constitutional symptoms (e.g., night sweats, pruritus, bone pain, fever, and fatigue) or splenomegaly. Consequently, it is of interest as to whether pomalidomide's pro-erythropoietic activity is mediated through modulation of expression of a specific cytokine repertoire that is distinct from JAK-1/2 inhibitors. Further, it is of interest as to whether the anemia response can be predicted by a specific pre-treatment cytokine

Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, Minnesota

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*Correspondence to: Ayalew Tefferi, MD, Division of Hematology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.
E-mail: tefferi.ayalew@ mayo.edu

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TABLE I. Cytokines Whose Plasma Levels are Abnormally Increased (or Decreased) Relative to Normal Controls

Cytokines (pg ml^{-1})	Controls median (range) $N = 35$	MF patients median (range) $N = 32$	P-value
IL-1b	4 (0–48.7)	18.4 (11.5–94.8)	<0.0001
IL-1RA	203.3 (2–419.3)	601.6 (57.2–3098.5)	<0.0001
IL-2	6.1 (0–27.6)	6.3 (4.4–39.0)	NS
IL-2R	216.9 (0–506.9)	777.8 (20.2–17354.8)	<0.0001
IL-4	7.2 (0–33.0)	32.3 (27.9–69.0)	<0.0001
EGF	32.7 (0–76.0)	18.4 (8.8–109.1)	NS
IL-6	0.6 (0–9.1)	11.3 (3.9–55.8)	<0.0001
IL-7	7.6 (0–51.5)	0.4 (0–34)	NS
IL-8	3.3 (0–17.7)	23.8 (0–1228.8)	<0.0001
IL-10	4.8 (2.3–50.6)	1.9 (0–20.6)	<0.0001
FGF	16.0 (0–66.8)	18.5 (3.9–148.9)	NS
IL-12	100.2 (34.8–181.5)	246.2 (17.8–875.6)	<0.0001
IL-13	0 (0–0)	0 (0–30.0)	0.03
IL-15	0 (0–38.0)	59.1 (9.3–223.7)	<0.0001
IL-17	0 (0–15.0)	6.7 (0–142.9)	0.0005
TNF-α	0 (0–15.0)	0 (0–30.0)	0.01
G-CSF	32.6 (0–373.2)	26.6 (0–336.7)	NS
INF-α	27.6 (0–95.9)	21.6 (0–130.7)	NS
INF-γ	5.5 (0–22.9)	0 (0–178.0)	<0.0001
GM-CSF	0 (0–172.0)	8.9 (5.0–63.7)	0.001
MIP-1a	0 (0–111.7)	47.1 (0–234.7)	<0.0001
MIP-1b	21.8 (4.4–91.0)	57.6 (0–419.2)	0.0005
HGF	129.4 (0–433.0)	347.9 (0–1413.2)	<0.0001
IP-10	21.9 (4–96.6)	96.6 (26.7–3332.9)	<0.0001
MIG	19.4 (0–86.3)	116.9 (31.0–315.9)	<0.0001
EOTAXIN	47.5 (7.6–155.5)	37.6 (8.5–164.1)	NS
RANTES	4130.4 (0–34346.7)	0 (0–3842.0)	0.002
MCP-1	173.1 (60.7–341.7)	318.5 (149.5–1725.9)	<0.0001
VEGF	1 (0–2.7)	2.2 (0–24.1)	0.004

Key: MF, myelofibrosis; IL, interleukin; TNF, tumor necrosis factor; G-CSF, granulocyte colony-stimulating factor; INF, interferon; MIP, macrophage inflammatory protein; HGF, hepatocyte growth factor; IP-10, IFN-γ inducible protein 10; MIG, monokine induced by IFN-γ; MCP-1, monocyte chemotactic protein-1; VEGF, vascular endothelial growth factor.

profile, and whether this effect is independent of *JAK2*V617F presence. The current manuscript examines the latter issue through a comprehensive analysis of circulating cytokine levels prior to pomalidomide treatment followed by correlation with anemia responses in myelofibrosis patients.

Methods

The current study was approved by the Mayo Clinic institutional review board. All patients provided informed written consent for study sample collection as well as permission for use in research. Inclusion to the current study required availability of archived plasma collected prior to treatment initiation in one of two clinical trials of pomalidomide treatment in myelofibrosis that accrued patients in Rochester, MN [15–17]. For patients treated on the Phase II randomized study, [17] all were confirmed (after unblinding at study end) to have received treatment on an arm that included pomalidomide.

Peripheral blood was collected under a Mayo Clinic protocol for patients with myeloid malignancies and standard procedures were followed to centrifuge samples at 4°C and store aliquots at −80°C. Concentrations of 31 plasma cytokines/chemokines were analyzed in duplicates using Multiplex Bead-based Luminex Technology (Invitrogen, Carlsbad, CA): interleukin (IL)-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-16, IL-17, epidermal growth factor (EGF), eotaxin, fibroblast growth factor-basic (FGF-b), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor (HGF), interferon (IFN)-α, IFN-γ, IFN-γ-inducible protein 10 (IP-10), monocyte chemotactic protein-1 (MCP-1), monokine induced by IFN-γ (MIG), macrophage inflammatory proteins (MIP)-1α, MIP-1β, regulated on activation normally T-cell expressed and secreted (RANTES), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF). Measurements were performed on a Luminex 200 analyzer (Luminex Corporation, Austin, TX) and resulting data were evaluated using Xponent (Luminex Corporation) software.

All statistical analyses considered clinical and laboratory parameters obtained at the time of screening prior to study entry, which coincided, in all instances, with time of plasma collection for cytokine analysis. Differences in the distribution of continuous variables between categories were analyzed by either Mann-Whitney (for comparison of two groups) or Kruskal-Wallis (comparison of three or more groups) test. Patient groups with nominal variables were compared by chi-square test. P values less than 0.05 were considered significant. The Stat View (SAS Institute, Cary, NC) statistical package was used for all calculations.

TABLE II. List of Cytokines Whose Plasma Levels in Patients With Myelofibrosis Were Associated With Anemia Response to Pomalidomide Therapy

	All subjects ($n = 32$)		*JAK2*V617F-positive subjects ($n = 25$)	
Cytokine[a]	Continuous[b]	≤ vs >3 SD[c]	Continuous[b]	≤ vs >3 SD[c]
IL-2R	0.009	NS ($n = 20$)	0.008	NS ($n = 17$)
IL-8	0.005	0.02 ($n = 19$)	0.02	0.07 ($n = 13$)
IL-15	0.07	NS ($n = 29$)	0.01	0.07 ($n = 23$)
MCP-1	0.04	0.05 ($n = 11$)	0.003	0.008 ($n = 10$)
VEGF	0.06	NS ($n = 14$)	0.06	NS ($n = 10$)

[a] In all instances, higher cytokine levels were associated with significantly lower rates of anemia response.

[b] Cytokine levels considered as continuous variables in the statistical analysis.

[c] Cytokine levels that exceeded 3 standard deviations (>3 SD) from the mean for normal controls were considered as significantly elevated; "n" refers to the number of patients for each cytokine with levels >3 SD.

Results

A total of 32 patients with myelofibrosis were identified (median age 66 years; range 36–86; 69% male), who, at the time of their screening visit prior to enrolling in a pomalidomide clinical trial had a plasma sample collected and stored for research purposes. Of these 24 (75%) had PMF, 5 (16%) post-ET MF, and 3 (9%) post-PV MF; 25 patients (78%) were *JAK2*V617F mutation-positive. Twenty six patients (81%) were red cell transfusion requiring prior to starting study treatment; 4 patients (13%) had a white blood cell count $>25 \times 10^9$/L, and 8 subjects (25%) a platelet count $<100 \times 10^9$/L; 19 patients (59%) had a circulating blast count of ≥1% and 7 patients (22%) an unfavorable karyotype [18].

Twenty one patients (66%) were enrolled in study MC078B and received pomalidomide monotherapy at a starting dose of 0.5 mg day^{-1} ($n = 17$) or 3.0 mg day^{-1} ($n = 4$); 11 (34%) patients were enrolled in study CC-4047-MMM-001 and treated on the following arms: pomalidomide 2 mg day^{-1} ($n = 4$); prednisone + pomalidomide 2 mg day^{-1} ($n = 5$), and prednisone + pomalidomide 0.5 mg day^{-1} ($n = 2$). At the time of cytokine analysis, 28 patients (88%) had discontinued study treatment; the median (range) of treatment cycles administered was 8 (1–32); 26 patients (81%) had received a minimum three cycles of treatment. Ten patients (31%) achieved anemia response per International Working Group for Myeloproliferative neoplasms Research and Treatment (IWG-MRT criteria), [19] all of whom harbored the *JAK2*V617F mutation; in other words, treatment response was 40% in *JAK2*V617F-positive patients.

Plasma cytokine levels measured in the study population were compared to those in normal controls ($n = 35$); significant differences ($P < 0.05$) were noted for 22 of the 30 cytokines assessed, and all were increased in this comparison except for IL-10, IFN-γ, and RANTES (Table I). In univariate analysis, increased levels of sIL-2R, IL-8, IL-15, MCP-1, and VEGF were significantly associated with inferior frequency of anemia response (borderline significance for IL-15 and VEGF; Table II). Increased levels of IL-8 and MCP-1 continued to show significant inverse correlation with anemia response when cytokine excess was defined as a plasma level exceeding three standard deviations (>3 SD) from the mean level in normal controls. Increased

levels of the aforementioned cytokines were not significantly correlated with presence of *JAK2*V617F. In univariate analysis, marked splenomegaly (palpable spleen size $\geq$10 cm) and increased serum LDH level were associated with poor response ($P = 0.02$ and 0.03, respectively), and with each other ($P = 0.02$), but not with *JAK2*V617F.

Since anemia responses were restricted to *JAK2*V617F mutation-positive patients, we repeated the cytokine-response correlation analysis in this population. Increased levels of four of the five previously identified cytokines (i.e., sIL-2R, IL-8, IL-15, and MCP-1) were found to be associated with a significantly inferior anemia response; when considering the binary definition of cytokine excess ($\leq$ or $>$3 SD), only MCP-1 maintained its significance (IL-8 and IL-15 were borderline significant) (Table II). Increased levels of sIL-2R ($P = 0.01$), IL-15 ($P = 0.06$), and MCP-1 ($P = 0.07$) were associated with marked splenomegaly.

Discussion

Recently, there has been a renewed interest on the role of dysregulated cytokines/chemokines in myelofibrosis. Much of this attention has been spurred by the availability of novel drugs (e.g., thalidomide, IMiDs, JAK inhibitors) whose therapeutic efficacy in myelofibrosis might be related to their immunomodulatory and/or anti-inflammatory properties. What has been missing however is a comprehensive approach that not only measures levels of a broad array of cytokines, but one that correlates baseline and post-treatment changes in cytokine levels with various response measures (i.e., anemia, splenomegaly, constitutional symptoms) as well as other longer-term outcome measures (i.e., leukemia-free survival and overall survival).

Recent observations during the course of myelofibrosis treatment with the JAK-1/2 inhibitor, INCB018424, revealed that, in a small cohort ($n = 23$), plasma levels of several pro-inflammatory/angiogenic cytokines were significantly elevated as compared to a control population at baseline; [12] after one cycle of treatment (at 25 mg BID dose level), cytokine levels were significantly suppressed; further, the degree of reduction in C-reactive protein (CRP), IL-1RA, MIP-1β, TNF-α, and IL-6 levels after six cycles of treatment correlated with the degree of improvement in constitutional symptoms. While these observations represent a significant advance in our understanding of cytokine-treatment response correlates, several aspects render this understanding incomplete: first, since anemia responses were rarely observed during INCB018424 treatment, the cytokine correlates of this response measure could not be assessed. Second, while 44% of subjects achieved a significant spleen response, the cytokine correlates of this response were not presented. Finally, it is unclear as to whether the pre-treatment cytokine levels were predictive of clinical response or any other clinical outcome measures.

The current study furthers our understanding of cytokine-treatment response correlates in myelofibrosis in two aspects: (i) it suggests the potential utility of a baseline plasma cytokine profile for predicting treatment response to certain therapeutic agents; and (ii) since the major efficacy of pomalidomide treatment lies in its ability to improve anemia, it specifically identifies the cytokine correlates of anemia response. We identified a specific repertoire of cytokines (sIL-2R, IL-8, IL-15, and MCP-1) whose baseline levels were predictive of anemia response; the predictive value appeared to be independent of *JAK2*V617F since their significance was preserved when the analysis was restricted to mutation-positive patients.

Finally, the aforementioned cytokine repertoire (i.e., sIL-2R, IL-8, IL-15, and MCP-1) revealed a remarkable overlap with the cytokines identified as being predictive of inferior survival in a much larger cohort of myelofibrosis patients (sIL-2R, IL-8, IL-12, and IL-15); [8] it is premature however to speculate at this point as to whether pomalidomide treatment will have any impact on the natural history of myelofibrosis on the basis of this observation.

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