CD14⁺ S100A9⁺ Monocytic Myeloid-derived Suppressor Cells and Their Clinical Relevance in Non-Small Cell Lung Cancer

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Rationale: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous family of myeloid cells that suppress T-cell immunity in tumor-bearing hosts. Their clinical relevance remains unclear.

Objectives: To identify subtypes of myeloid-derived suppressor cells in patients with non-small cell lung cancer (NSCLC) and their clinical relevance.

Methods: CD11b⁺CD14[−] and CD11b⁺CD14⁺ cells, determined and phenotyped by fluorescence-activated cell sorter analysis, in the peripheral blood mononuclear cells (PBMCs) of treatment-naïve patients with advanced NSCLC were correlated with clinical data. T-cell activation in response to CD3/CD28 costimulation was determined by carboxy-fluorescein diacetate succinimidyl ester (CFSE) staining and ELISA analysis of IFN- γ . The percentage of CD11b⁺CD14⁺S100A9⁺ cells in PBMCs was correlated with and tested as a predictor for treatment response in a cohort of patients prospectively receiving first-line cisplatin-based chemotherapy.

Measurements and Main Results: Patients with NSCLC had a significantly higher ratio of CD11b⁺CD14⁺ cells than healthy subjects, which was correlated with poor performance status and poor response to chemotherapy. The depletion of these cells in the PBMC reversed the suppression of CD8⁺ and CD4⁺ T cells. Isolated CD11b⁺CD14⁺ cells suppressed CD8⁺ T-cell proliferation and IFN- γ production, and the former effect was attenuated by the inducible nitric oxide synthase (iNOS) inhibitor aminoguanidine hydrochloride, arginase inhibitor *N*-hydroxy-nor-L-arginine (nor-NOHA), and blocking antibodies for IL-4R α ⁺ and IL-10. CD11b⁺CD14⁺ cells were monocyte-like, expressing CD33⁺, CD15^{−/low}, IL-4R α ⁺, and S100A9⁺ and producing iNOS, arginase, and several cytokines. The ratio of S100A9⁺ cells positively correlated with the suppressive ability of the CD11b⁺CD14⁺ cells, was associated with poor response to chemotherapy, and predicted shorter progression-free survival.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Subpopulations of myeloid-derived suppressor cells (MDSCs) have been described in human cancers. Their clinical relevance is not clear.

What This Study Adds to the Field

CD11b⁺/CD14⁺ MDSCs are the predominant type of MDSCs and have clinical relevance in patients with non-small cell lung cancer. S100A9 can be used as a marker of these cells, several of which are associated with poor response to chemotherapy and could be a predictor for shorter progression-free survival.

Conclusions: CD14⁺S100A9⁺ inflammatory monocytes in patients with NSCLC are a distinct subset of MDSCs, which suppress T cells by arginase, iNOS, and the IL-13/IL-4R α axis. The amount of these inflammatory monocytes is associated with poor response to chemotherapy. Clinical trial registered with www.clinicaltrials.gov (NCT 01204307).

Keywords: non-small cell lung cancer; myeloid-derived suppressor cell; S100A9; cancer immunity

Evading immune destruction is an emerging hallmark of cancer (1). In addition to generating weakly immunogenic cancer cells through the immunoediting process, cancer cells can evade immune destruction by disabling components of the immune system, particularly CD8⁺ cytotoxic T lymphocytes, CD4⁺ Th1 helper T cells, and natural killer (NK) cells (1). This final mechanism, NK cells, partially explains the disappointing clinical results from immunotherapy (2).

Myeloid-derived suppressor cells (MDSCs) are heterogeneous populations of myeloid cells found in tumor-bearing mice and patients with various forms of cancer that actively inhibit the function of NK and T cells (3–5). In murine models, MDSCs expressing CD11b and Gr-1 accumulate and expand in the lymphoid organ, bone marrow, peripheral blood, and tumor microenvironment. These cells promote tumor progression by suppressing cellular immunity against tumors (6, 7). At least two subsets of MDSCs have been described based on the expression levels of LY6C or LY6G: monocytic MDSCs and granulocytic MDSCs (5, 8). In a murine model of colon carcinoma, a distinct population of CD11b⁺ IL-4 receptor α ⁺ (CD11b⁺IL-4R α ⁺) inflammatory-type monocytes was shown to confer a major suppressive function on CD8⁺ T cells (9). However, the characterization of human MDSCs is hampered because of the absence of a homologous Gr-1 gene in humans. Nonetheless, MDSCs expressing CD11b⁺CD14[−]CD15⁺ with polymorphonuclear

(Received in original form April 9, 2012; accepted in final form August 21, 2012)

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Supported by grants to H.-P.K. and K.-Y.L. from Chang Gung Memorial Hospital (CMRPG300181).

Author Contributions: Conception and design, P.-H.F., K.-Y.L., T.-Y.L., F.-T.C., C.-S.K., C.-T.Y., S.-M.L., C.-H.W., C.-L.C., C.-D.H., and H.-P.K.; analysis and interpretation, P.-H.F., K.-Y.L., Y.-L.C., T.-Y.L., F.-T.C., C.-S.K., C.-T.Y., S.-M.L., C.-H.W., C.-L.C., C.-D.H., and H.-P.K.; and drafting the manuscript for important intellectual content, P.-H.F., K.-Y.L., Y.-L.C., T.-Y.L., F.-T.C., C.-S.K., C.-T.Y., S.-M.L., C.-H.W., C.-L.C., C.-D.H., and H.-P.K.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 186, Iss. 10, pp 1025–1036, Nov 15, 2012

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Originally Published in Press as DOI: 10.1164/rccm.201204-0636OC on September 6, 2012

Internet address: www.atsjournals.org

morphology can be found in patients with metastatic renal cell carcinoma (10) and non-small cell lung cancer (NSCLC) (11). Recently, a report showed that there was an expansion in the CD14⁺ mononuclear population of MDSCs in patients with colon cancer and that the presence of IL-4R α ⁺ cells in these cells was positively correlated with their inhibitory activity (12).

MDSCs inhibit the function of NK and T cells through various mechanisms. MDSCs suppress T-cell proliferation through the up-regulation of arginase 1, which depletes T-cell proliferation-required L-arginine (5, 13). In addition, MDSCs may also suppress T cells through the induction of FOXP3⁺ T regulatory cells by the secretion of IL-10 and IFN- γ (14). In addition, granulocytic MDSCs suppress T cells through high levels of reactive oxygen species (ROS) and peroxynitrite, whereas monocytic MDSC suppress T cells mostly by nitric oxide (5, 8). CD14⁺HLA-DR^{-/low} MDSCs have been identified in patients with metastatic melanoma and hepatocellular carcinoma and suppress T-cell proliferation by the up-regulation of arginase 1 and oxidative stress (3, 15–17). The effectiveness of distinct subsets of MDSCs and their clinical relevance has not yet been well evaluated.

MDSCs are recruited from bone marrow to peripheral blood by tumor-derived factors, including growth factors and inflammatory cytokines or chemokines (18), and these tumor-derived factors profoundly affect MDSCs mobilization and activation (19). Recently, the myeloid-related protein S100A9, which belongs to the damage-associated molecular pattern molecules, has been implicated in MDSCs accumulation and expansion in tumor-bearing mice (20). The production of S100A8/A9 in MDSCs, which serves as an autocrine feedback loop, mediates migration and sustains the accumulation of MDSCs by activation of the nuclear factor- κ B pathway (21). The presence of S100A9 in the immunosuppression activity of MDSC and its clinical relevance has not yet been demonstrated in human cancers.

This report demonstrates similar effectiveness of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ MDSCs in the suppression of CD8⁺ T-cell proliferation in patients with NSCLC. However, CD11b⁺CD14⁺ MDSCs are more clinically relevant in terms of performance status and clinical response to platinum-based chemotherapy. The mechanism underlying the suppressive function of the CD11b⁺CD14⁺ subpopulation and the role of S100A9 in this prospective setting is also well characterized.

METHODS

Subjects

In the first part of this cross-sectional analysis, 52 treatment-naive stage IV or locally advanced patients with NSCLC (male/female = 33/19; mean age, 59.5 yr) and 17 healthy subjects (male/female = 9/8; mean age, 36.2 yr) were recruited for MDSC studies. Among the patients with NSCLC, 40 had adenocarcinoma, six had squamous cell carcinoma, and six had nonclassified NSCLC. In addition, 40 patients who had adequate performance (World Health Organization performance status [PS] $\leq$ 1) and had received cisplatin-based chemotherapy as the first-line treatment in a randomized prospective trial were recruited to test the predictive role of MDSCs. Of these 40 patients, 17 were randomized to receive cisplatin (75 mg/m²)/pemetrexed (500 mg/m²) every 3 weeks, and 23 were to receive cisplatin (75 mg/m²)/docetaxel (30 mg/m² on Day 1 and Day 8) every 3 weeks. All of the patients received chemotherapy for four to six cycles or until disease progression. The Institutional Review Board of Chang Gung Memorial Hospital approved this prospective observational study (No. 98–3692B), and informed consent was obtained from all subjects. The second part of this study involved the validation of the significance of S100A9. Patients were recruited from a multicenter prospective study (NCT01204307). Their data are summarized in Table 1.

Tumor response was evaluated using computed tomography according to the Response Evaluation Criteria in Solid Tumors criteria and

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS

	All Cases	$\leq$ 23%	>23%
Sex (male/female)	24 (14/10)	14 (8/6)	10 (5/5)
Age, yr (mean $\pm$ SD)	57.6 $\pm$ 12	60.3 $\pm$ 11	53.9 $\pm$ 13
Cell type			
Adenocarcinoma	23	13	10
Non-small cell lung cancer	1	1	0
Smoking status			
Never smoking	14	9	6
Ever-smoker	6	5	4
First-line treatment			
Pemetrexed + cisplatin	9	3	6
Taxotere + cisplatin	15	11	4
Treatment response			
Partial response	9	7	2
Stable disease	10	6	4
Progress disease	5	1	4

classified as complete response, partial response (PR), stable disease (SD), or progressive disease (PD). Clinicopathologic information, including patient characteristics, treatment, clinical staging results, histologic subtype and survival data, was recorded.

Isolation and Depletion of Cells

Peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll Paque (GE Healthcare, Bio-science AB, Uppsala, Sweden) density gradient method. CD11b⁺CD14⁻ and CD11b⁺CD14⁺ cells were immunomagnetically isolated from PBMCs by sequentially using the anti-CD14 and anti-CD11b MACS magnetic sorting system (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions. The purity of the two subsets of cells was greater than 90% and greater than 95%, respectively, as confirmed by flow cytometry. CD14⁺HLA-DR^{-/low} cells were immunomagnetically enriched from PBMCs by anti-HLA-DR negative selection, followed by anti-CD14 positive selection with greater than 85% purity.

Antibodies and Flow Cytometry

Antibody details are provided in Table E1 in the online supplement. PBMCs or specific cells were stained according to the manufacturer's recommendations. To measure the expression of CD11b, CD14, CD15, CD33, IL-4R α , RAGE, HLA-DR, CD4, and CD8 in specific types of cells, 2×10^5 cells were incubated for 10 minutes at 4°C with specific mouse antihuman monoclonal antibodies conjugated with fluorochromes (fluorescein isothiocyanate, PE or PE-Cy5 or PE/Alexa; R&D Systems, Minneapolis, MN). For intracellular staining, the cells were permeabilized by BD fluorescence-activated cell sorter permeabilizing solution (BD Biosciences, San Jose, CA) and stained with antibodies specific for inducible nitric oxide synthase (iNOS), arginase 1, S100A8, or S100A9. The data from 10,000 events were analyzed with FlowJo software (TreeStar, Inc., Ashland, OR). To understand the influence of the percentage of CD11b⁺CD14⁺ cells in PBMCs from patients with NSCLC, the number of CD11b⁺CD14⁺ cells was calculated in gated nonlymphocytic mononuclear cells and transformed into the percentage of PBMCs. CD11b⁺CD14⁺S100A9⁺ cells were evaluated in the same manner.

Oxidative Stress Measurement

The intracellular oxidant intensity was determined by fluorescence-activated cell sorter analysis of 5-(and -6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate-acetyl-ester (DCFDA; Invitrogen, Carlsbad, CA), which is metabolized to fluorescent 2',7'-dichlorofluorescein (DCF) upon oxidation, as previously described (22). The detailed methods are available in the online supplement.

Activation of T Cells

The proliferation assay of T cells in PBMCs with or without depletion of MDSCs was performed using carboxy-fluorescein diacetate succinimidyl

ester (CFSE) assay as previously described (10). The detailed methods are available in the online supplement. In some experiments, subunits of MDSCs (5×10^5 cells) (e.g., CD11b⁺CD14⁻, CD11b⁺CD14⁺, or CD14⁺HLA-DR^{-low} MDSCs) were added to the CD11b and CD14 double-depleted PBMCs (5×10^5 cells) and used as effector cells at a final volume of 500 μ l to show their inhibition activity. T-cell activation was determined by an ELISA of IFN- γ production in cultured supernatants 48 hours after stimulation (BD Biosciences).

To study the suppression mechanisms used by CD11b⁺CD14⁺ MDSCs, the proliferation of CD8⁺ T cells was determined in the presence of CD11b⁺CD14⁺ cells with 30 minutes of pretreatment with pharmacologic inhibitors for iNOS (aminoguanidine hydrochloride [AG], 500 μ M; Tocris Bioscience, Ellisville, MO); arginase 1 (2S-amino-4-[(hydroxyamino) iminomethyl] amino]-butanoic acid dihydrochloride hydrate; nor-NOHA, 10 μ M; Cayman Chemical, Ann Arbor, MI); Stat3 (Tyrphostin AG490, 10 μ M; Sigma-Aldrich, Inc. St. Louis, MO); the oxidant scavenger *N*-acetylcysteine (10 mM, Sigma-Aldrich); and neutralizing antibodies for IL-4R (100 ng/ml; R&D Systems), IL-10 (10 μ g/ml; R&D Systems), and IgG (1 μ g/ml; DAKO, Glostrup, Denmark).

ELISA

CD11b⁺CD14⁺ cells (5×10^5 cells) from patients with NSCLC or healthy donors were cultured in 24-well plates in RPMI (500 μ l) for 24 hours. The levels of IL-6, IL-10, IL-8, IL-13, tumor necrosis factor (TNF)- α , transforming growth factor- β 1, hepatocyte growth factor (HGF), and insulin-like growth factor in the cultured supernatant were quantified by commercially available ELISA kits (R&D Systems) according to the manufacturer's specifications. All samples were run in batches to minimize interassay variability, assayed in duplicate, and quantitated using a standard curve.

In Vitro Migration Assay

The *in vitro* migration assay was evaluated in 24-well plates with transwell polycarbonate permeable supports (8 μ m) (Corning Incorporated, Corning, NY). First, 3×10^4 CFSE-labeled CD11b⁺CD14⁺ cells from normal control (NC) or patients with NSCLC were plated in 200- μ l RPMI with 10% fetal bovine serum in the upper chambers, whereas 6×10^4 A549 cells were plated in the bottom chambers with 800- μ l Dulbecco's modified Eagle medium and 10% fetal bovine serum. Next, 1 ng/ml S100A9 human recombinant protein (Abnova Corporation, Taipei, Taiwan) or 10 μ g monoclonal antihuman RAGE blocking antibody (R&D Systems) was added to the upper chambers and then cultured at 37°C with 5% CO₂ overnight. The cells were removed from the upper surface of the filter by gentle rubbing with a cotton-tipped swab, and the filters were mounted on microscope slides. The cells that had migrated through the pores were counted in four randomly chosen fields under a fluorescence microscope.

A549-MDSC Coculture and MTT Assay

A549 cells (2×10^3 cells) (bottom chamber) were cocultured with or without equal numbers of CD14⁺ cells from patients with NSCLC (upper chamber) in 24-well plates with transwell polycarbonate permeable supports (0.45 μ m) (Corning Incorporated) of small size to prevent the migration of cells. After overnight incubation at 37°C with 5% CO₂ in the presence or absence of RAGE blocking antibody (10 μ g/ml) or IgG control (1 μ g/ml, DAKO), cisplatin was added into the bottom chamber at 0, 50, and 100 μ M. After 4 days of culture, A549 cell viability was determined by an MTT assay (Promega, Madison, WI) according to the manufacturer's instructions.

Statistical Analysis

The categorical variances between groups were assessed by Kruskal-Wallis analysis. The analysis of continuous variance between groups was performed using the Mann-Whitney *U* test or Wilcoxon signed-rank test for unpaired or paired data. The analysis of continuous variance of more than three groups was performed using analysis of variance. The relationships between two parameters were investigated using Spearman rank correlation test. Progression-free survival (PFS) was analyzed and

defined as the period from the start of treatment to documented progression. Survival curves were estimated by the Kaplan-Meier method, whereas the log-rank test was used to compare the patient survival times per group. GraphPad Prism (version 5.0; GraphPad Software, San Diego, CA) was used for all statistical analyses, and statistical significance was defined as *P* less than 0.05.

RESULTS

Increased CD11b⁺CD14⁺ Population in the PBMCs of Patients with NSCLC and Its Clinical Relevance

The number of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells was increased in nonlymphocytic mononuclear cells (Figure 1A) (see Figures E1A and E1B). To elucidate the clinical relevance of these two subsets of myeloid cells in patients with NSCLC, the percentage of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells in PBMC was calculated. The percentage of CD11b⁺CD14⁺ ($21.4 \pm 1.63\%$; *n* = 37) and CD11b⁺CD14⁻ ($6.53 \pm 1.77\%$; *n* = 37) cells was higher in patients with NSCLC than normal healthy donors (Figure 1B) ($11.9 \pm 1.29\%$, *n* = 17, *P* < 0.001; and $2.21 \pm 0.89\%$, *n* = 17, *P* < 0.001, respectively). Patients with poor performance status (PS $\geq$ 2) had a significantly higher percentage of CD11b⁺CD14⁺ cells ($40.9 \pm 5.1\%$; *n* = 6; *P* < 0.001), but not of CD11b⁺CD14⁻ cells ($11.6 \pm 5.9\%$; *n* = 9; *P* > 0.05) compared with patients with PS less than two ($17.4 \pm 1.6\%$, *n* = 9; and $3.5 \pm 1.8\%$, *n* = 3, respectively) (Figure 1C). The percentage of CD11b⁺CD14⁺ cells but not CD11b⁺CD14⁻ cells was associated with treatment response to platinum-based chemotherapy ($14.7 \pm 0.8\%$, *n* = 10; $20.6 \pm 2\%$, *n* = 9, and $27.2 \pm 4.5\%$, *n* = 8, for PR, SD, and PD, respectively; *P* < 0.001) (Figure 1D).

CD11b⁺CD14⁺ Cells Are CD15⁻CD33⁺IL-4R α ⁺ Monocytic Cells with Inflammatory Characteristics

The CD11b⁺CD14⁺ cells from patients with NSCLC, similar to those from healthy donors, expressed low/negative CD15 and positive CD33, whereas NSCLC and normal control CD11b⁺CD14⁻ cells expressed high CD15 and low/negative CD33 (Figures 2A and 2B). The monocytic morphology of these CD11b⁺/CD14⁺ cells was confirmed by microscopy (Figure 2C). Most of the NSCLC CD11b⁺CD14⁺ cells ($87.3 \pm 3.8\%$; *n* = 4) expressed IL4R α ⁺ at a higher ratio compared with the ratio of normal CD11b⁺CD14⁺ cells ($49.5 \pm 9\%$; *n* = 6; *P* < 0.05) and that of NSCLC CD11b⁺CD14⁻ cells ($5.6 \pm 3.8\%$; *n* = 6; *P* < 0.05) (Figures 2A and 2B). However, the mean expression levels of IL4R α ⁺ in CD11b⁺CD14⁺ cells were almost identical between the patients with NSCLC and normal control subjects (see Figure E2). There was a trend of fewer HLA-DR⁺ cells with lower intensity in the CD11b⁺CD14⁺ cells from patients with NSCLC compared with those from healthy subjects (see Figure E2). A similar trend of fewer HLA-DR⁺ cells was also observed in CD11b⁺CD14⁻ cells from patients with NSCLC compared with those of healthy donors (Figure 2B).

CD11b⁺CD14⁺ cells from patients with NSCLC produced higher levels of proinflammatory cytokines (e.g., TNF- α , IL-8, IL-10, IL-13, and HGF), which supports the hypothesis that they possess inflammatory characteristics (Figure 2D).

CD11b⁺CD14⁺, Not Restricted to HLA-DR^{-low} Cells, and CD11b⁺CD14⁻ Cells Inhibit T-Cell Activation

We next examined the immunosuppressive activities of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells on T cell proliferation, as demonstrated by the attenuation of CFSE staining, in PBMCs with CD3/CD28 stimulation. CD8⁺ T-cell proliferation was

Figure 1. Clinical relevance of CD11b⁺CD14⁺ cells in patients with non-small cell lung cancer (NSCLC). (A) Representative dot plots of the peripheral blood mononuclear cells (PBMCs) of patients with NSCLC (CA) and normal healthy donors (NC). Nonlymphocyte mononuclear cells were gated, and CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells were analyzed by flow cytometry. (B) Ratio of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells in the PBMCs of normal controls (n = 17) and patients with NSCLC (n = 37). (C) The number of CD11b⁺CD14⁺ cells was increased in poor-performance patients. PS < 2, n = 9; PS ≥ 2, n = 6. (D) The number of CD11b⁺CD14⁺ cells was increased in patients with progressed disease after treatment. PR, n = 10; SD, n = 9; PD, n = 8. The data are the mean ± SEM; *P < 0.05; ***P < 0.001. FSC = forward scatter; PD = progressive disease; PR = partial response; PS = performance status; SD = stable disease; SSC = side scatter.

suppressed in the patients with NSCLC but not the healthy donors (see Figure E3). This proliferation was restored after the depletion of either CD11b⁺ or CD14⁺ cells (Figure 3A). In addition, CD4⁺ T cells were similarly suppressed (data not shown).

To confirm the suppressive ability of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells, both cells were isolated and cocultured with equal amounts of CD11b⁻ and CD14⁻ double-depleted autologous PBMCs, which served as effector cells. CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells from patients with NSCLC robustly suppressed the CD8⁺ T cells in the effector cells at a 1:1 ratio (Figure 3B). The suppressive ability was similar between these two suppressor cells, as demonstrated by the proportional decrease in the ratio of the suppressor cells in the coculture systems. The suppressive ability of both groups of cells remained significant at a ratio of 1:2 MDSC to effector cells (Figure 3C). Moreover, both suppressor cells inhibited IFN-γ production by CD8⁺ T cells to a similar extent (28.7 ± 9.1% by CD11b⁺CD14⁻, n = 4, P < 0.05 and 17.7 ± 8.3% by CD11b⁺CD14⁺, n = 4, P < 0.05 compared with the effector cells only, n = 4) (Figure 3D). In contrast, CD11b⁺CD14⁺ cells from healthy donors showed no suppressive ability on CD8⁺ T-cell proliferation or IFN-γ production (Figures 3C and 3D) (see Figure E4). The CD11b⁺CD14⁻ cells in the PBMCs of healthy donors were limited, and not enough cells were isolated to complete the proliferation and IFN-γ studies.

To confirm whether the suppressive function was restricted to the HLA-DR^{-low} population, CD14⁺HLA-DR^{-low} cells were isolated (see Figure E5). Although CD11b⁺CD14⁺ and CD14⁺HLA-DR^{-low} cells suppressed CD8⁺ T-cell proliferation, the CD14⁺HLA-DR^{-low} cells had a weaker suppressive ability (66 ± 8.7%; n = 5) than the CD11b⁺CD14⁺ cells (23 ± 5.4%; n = 5; P < 0.05), which were a mixture of CD14⁺HLA-DR^{high} and CD14⁺HLA-DR^{-low} cells (Figure 3E). Thus, the suppressive

function of CD11b⁺CD14⁺ MDSCs was not restricted to HLA-DR^{-low} cells.

CD11b⁺CD14⁺ MDSC Suppression of T-Cell Proliferation via iNOS, Arginase 1, and the IL-13/IL-4Rα Pathway

A higher proportion of NSCLC CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells expressed iNOS (80 ± 4.8%, n = 6 and 87 ± 3.6, n = 5, respectively; both P < 0.05) and arginase 1 (87 ± 3%, n = 5 and 88 ± 5.6%, n = 3, respectively; both P < 0.05) compared with normal cells (43.6 ± 7.8%, n = 5 and 34 ± 15%, n = 4, respectively) (Figures 4A and 4B). The expression levels of these proteins, as determined by mean fluorescence intensity (MFI), were also higher in NSCLC CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells (iNOS, 196 ± 30.8, n = 5 and 355 ± 107, n = 4, respectively; and arginase 1, 99 ± 14.7, n = 5 and 319 ± 43, n = 3, respectively; all P < 0.05) compared with healthy subjects (28 ± 5.9, n = 5 and 36 ± 14, n = 4, respectively) (see Figure E6). However, there was no percentage difference in intracellular ROS production determined by fluorescence-activated cell sorter measurement of 2',7'-dichlorofluorescein (DCF) between the healthy donor cells and NSCLC CD11b⁺CD14⁺ cells, although the NSCLC CD11b⁺CD14⁻ cells had lower MFI of DCF than the CD11b⁺CD14⁺ cells of NSCLC and healthy donors (Figure 4B) (see Figure E6). Functionally, T-cell suppression was prevented by pretreatment with the iNOS inhibitor AG and arginase 1 inhibitor nor-NOHA but not the ROS scavenger N-acetylcysteine, STAT3 inhibitor AG490, or anti-RAGE monoclonal antibody (Figure 4C). In addition, pretreatment with the neutralizing antibodies for IL-4Rα or IL-10 also restored T-cell proliferation (Figure 4C). These results suggest that iNOS, arginase 1, IL-13/IL-4Rα axis, and IL-10 but not STAT3/ROS mediate the suppressive effect of CD11b⁺CD14⁺ monocytic MDSCs on T-cell proliferation.

Figure 2. Surface markers of CD11b⁺CD14⁺ cells. (A) Representative histogram of various surface markers expressed on CD11b⁺/CD14⁺ and CD11b⁺/CD14⁻ cells from normal subjects (NC) and patients with non-small cell lung cancer (NSCLC) (CA) analyzed by flow cytometry. The data for CD11b⁺/CD14⁻ myeloid-derived suppressor cells (MDSCs) from patients with NSCLC are also shown. *Gray lines*, IgG control; *black lines*, surface marker staining as indicated. (B) Column bar graph analysis of the data as in (A) are presented as percentage of positively stained cells. *Open bars*, normal CD11b⁺CD14⁺ cells; *dark gray bars*, NSCLC CD11b⁺CD14⁺ cells; *light gray with dots*, normal CD11b⁺CD14⁻ cells; *dark gray with dots*, NSCLC CD11b⁺CD14⁻ cells; n = 6. (C) Microscopy analysis of the morphology of CD11b⁺CD14⁻ and CD11b⁺CD14⁺ cells from patients with NSCLC. (D) ELISA analysis of cytokine profiles in the cultured supernatants of CD11b⁺CD14⁺ MDSCs isolated from patients with NSCLC. CD11b⁺/CD14⁺ cells from patients with NSCLC produced higher levels of tumor necrosis factor (TNF)-α (2.6 ± 0.4 ng/ml; n = 3; P < 0.05), IL-8 (137.9 ± 7.4 ng/ml; n = 3; P < 0.05), IL-10 (21.3 ± 5.7 ng/ml; n = 3; P < 0.05), IL-13 (89 ± 46.3 pg/ml; n = 3; P < 0.05) and hepatocyte growth factor (HGF) (110 ± 14 pg/ml; n = 3; P < 0.05) compared with normal subjects (0.8 ± 0.1 ng/ml, 36.4 ± 1.6 ng/ml, 5.3 ± 0.3 ng/ml, and 11.6 ± 0.5 pg/ml, 42.9 ± 1.4 pg/ml, respectively). n = 3. The data are the mean ± SEM; *P < 0.05.

Figure 3. Immunosuppressive activity of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells. (A) *Upper panel*, representative histograms of flow cytometry analysis of CD8 T-cell proliferation in peripheral blood mononuclear cells (PBMCs) and CD11b⁻ depleted or CD14⁻ depleted PBMCs from healthy donors (NC) and patients with non-small cell lung cancer (NSCLC) (CA). *Lower panel*, effects of CD11b⁻ and CD14⁻ depletion on CD8 T-cell proliferation. Column bar graph analysis of the data as in the upper panel. CD8⁺ T-cell proliferation; empty bar, healthy donor (n = 6); solid bar, patients with NSCLC (n = 6). The data are the mean $\pm$ SEM and are presented as the percentage of maximal T-cell proliferation. (B) *Upper panel*, representative histograms of T-cell proliferation after adding CD11b⁺CD14⁺ or CD11b⁺CD14⁻ cells into equal amount autologous effector cells (CD11b-depleted PBMCs). *Lower panel*, column bar graph analysis of the data as in the upper panel. Open bar, normal control (n = 3); solid bar, patients with NSCLC, (n = 6). The data are the mean $\pm$ SEM and are presented as the percentage of maximal T-cell proliferation. (C) Suppressive ability of CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells from patients with NSCLC. CD11b⁺CD14⁺ or CD11b⁺CD14⁻ myeloid-derived suppressor cells (MDSCs) from patients with NSCLC and CD11b⁺CD14⁺ cells from healthy donors were cocultured with carboxy-fluorescein diacetate succinimidyl ester (CFSE)-labeled autologous effector cells in variable proportions (1:4, 1:2, or 1:1), and T-cell proliferation was analyzed. The data were from three independent experiments. (D) Effects of NSCLC CD11b⁺CD14⁺ and CD11b⁺CD14⁻ cells on CD3/CD28-stimulated IFN- γ production in the cultured supernatants of effector cells analyzed by ELISA. n = 4 independent experiments. The data are the mean $\pm$ SEM and are presented as the percentage of maximal IFN- γ production. (E) Proliferation assay analysis of CD8 T cells in CD11b-depleted PBMCs (effector cells) in the presence or absence of CD11b⁺CD14⁺ cells and CD14⁺HLA-DR^{low}-enriched cells. n = 5. The data are the mean $\pm$ SEM and are presented as the percentage of maximal CD8⁺ T-cell proliferation; * $P < 0.05$.

Figure 4. CD11b⁺CD14⁺ myeloid-derived suppressor cell (MDSC) suppression of T-cell proliferation is dependent on inducible nitric oxide synthase (iNOS), arginase 1, and the IL-13/IL-4R α pathway. (A) Representative histograms of flow cytometry analysis of iNOS, arginase 1, and intracellular oxidative stress as determined by the DCFH method in CD11b⁺CD14⁺ cells from healthy donors (NC) and patients with non-small cell lung cancer (NSCLC) (CA) and CD11b⁺CD14⁻ cells from patients with NSCLC. Gray lines, IgG control; black lines, iNOS, arginase 1 or 2',7'-dichlorofluorescein (DCF) staining. (B) Open bar, CD11b⁺CD14⁺ cells from health donor; black bar, NSCLC CD11b⁺CD14⁺; gray bar, NSCLC CD11b⁺CD14⁻. Column bar graph analysis of the percentage of positive staining cells as in (A). The data are represented as the mean $\pm$ SEM, n = 5; *P < 0.05. (C) Effects of variable pharmacologic inhibitors, neutralizing antibodies, and IgG control on the CD14⁺ MDSC suppression of T-cell proliferation. The data are the mean $\pm$ SEM and are presented as the percentage of maximal T-cell proliferation. n = 6, *P < 0.05.

S100A9 as Marker of CD11b⁺CD14⁺ MDSCs

There were higher proportions of S100A8⁺ ($76.1 \pm 3.4\%$; $n = 6$; $P < 0.05$) and S100A9⁺ ($82.9 \pm 6.4\%$; $n = 7$; $P < 0.05$) cells in NSCLC CD11b⁺CD14⁺ cells compared with those in the normal control cells ($49.5 \pm 8.2\%$ and $39.6 \pm 4.9\%$, respectively; both $n = 5$). In addition, the mean expression level of S100A9 was remarkably higher in patients with NSCLC (2141 ± 418 ; $n = 6$; $P < 0.05$) than healthy subjects (685 ± 434 ; $n = 4$). There was a trend of increased S100A8 expression in patients with NSCLC (998 ± 380 ; $n = 6$) compared with the level in healthy subjects (387 ± 162 ; $n = 5$; $P > 0.05$) (Figures 5A and 5B). The percentage of S100A9⁺ cells in NSCLC CD11b⁺CD14⁺ cells strongly correlated with their ability to suppress T cells (Figure 5C) ($n = 8$; Spearman $r = -0.85$; $P = 0.01$), which was not observed in the healthy donors (see Figure E7). This relationship remained when S100A9 was expressed as MFI (Figure 5D) ($n = 6$; Spearman $r = -0.89$; $P = 0.033$), suggesting that S100A9 can be used as a marker of CD11b⁺CD14⁺ MDSCs.

Clinical Relevance of CD11b⁺CD14⁺S100A9⁺ MDSCs

To elucidate the clinical relevance of CD11b⁺CD14⁺S100A9⁺ MDSCs, 24 patients with NSCLC receiving cisplatin-based chemotherapy were longitudinally followed-up (Table 1). Patients with PD had higher ratio of CD11b⁺CD14⁺S100A9⁺ cells ($39.2 \pm 7.1\%$) in the PBMCs compared with those with PR ($17.9 \pm 1.7\%$; $P < 0.05$) and SD ($19.4 \pm 4\%$; $P < 0.05$) (Figure 6A). The percentage of CD11b⁺CD14⁺ and CD11b⁺CD14⁺S100A9⁺ cells (Spearman $r = -0.83$, $n = 24$, $P < 0.0001$ and Spearman $r = -0.58$, $n = 24$, $P = 0.001$, respectively) (Figures 6A and 6B) but not of CD11b⁺CD14⁺IL-4Rα⁺ cells (Spearman $r = -0.02$; $n = 14$; $P = 0.93$) (see Figure E8) negatively correlated with PFS. CD11b⁺CD14⁺S100A9⁺ cells had a stronger correlation than CD11b⁺CD14⁺ cells.

To test whether S100A9 could be a predictor for chemotherapy treatment efficacy, patients were divided into high and low CD11b⁺

CD14⁺S100A9⁺ groups (Table 1) using the median value of 20% as the cut-off level. Patients in the low CD11b⁺CD14⁺S100A9⁺ group had significantly longer PFS than those in the other groups (9.2 vs. 3 mo; HR, 0.06; $P < 0.001$, by log-rank test) (Figure 6D). A similar result was also observed when using CD11b⁺CD14⁺ cells as a marker (9 vs. 2.9 mo; HR, 0.30; $P = 0.03$, by log-rank test) (Figure 6E), although it was a weaker predictor by HR.

Functional Roles of S100A9 in CD14⁺ MDSCs

To evaluate the role of S100A9 in the migration of CD14⁺ cells toward tumors, an *in vitro* transwell migration assay was performed in which CFSE-labeled CD14⁺ cells were seeded in the top chambers and A549 cells in the bottom ones. When S100A9 was added to the upper chamber, normal CD14⁺ cell migration was enhanced in a concentration-dependent manner (complete data not shown) (85.3 ± 27.2 cells with S100A9 1 ng/ml; $n = 4$; $*P < 0.05$) (Figures 6F and 6G). NSCLC CD14⁺ cells in the absence of exogenous S100A9 showed an augmented migratory ability compared with normal CD14⁺ cells (112.5 ± 32.8 vs. 14.5 ± 2 cells/field; $n = 4$; $*P < 0.05$) (Figures 6F and 6G). This augmented migratory ability of NSCLC CD14⁺ cells was markedly inhibited by 10 μg of a RAGE blocking antibody (10 μg/ml) (13.5 ± 2.1 cells/field; $n = 4$; $P < 0.05$) (Figures 6F and 6G). Confocal microscopy confirmed an increased interaction between S100A9 and RAGE on the cell membrane of NSCLC CD14⁺ cells (see Figure E9).

When cocultured with A549 cells, the CD14⁺ MDSCs from patients with NSCLC significantly attenuated the cisplatin-induced cytotoxicity of A549 cells at 50 μM and 100 μM (Figure 6H). A RAGE blocking antibody (10 μg/ml) failed to reverse the effect of NSCLC MDSC (see Figure E10).

DISCUSSION

This study characterized CD11b⁺CD14⁺ cells as a subpopulation of MDSCs in the PBMCs of patients with NSCLC. The proportion of these monocytic cells in the PBMCs corresponded with the

Figure 5. S100A9 as a marker of CD11b⁺CD14⁺ myeloid-derived suppressor cells (MDSCs). (A) Representative histograms of flow cytometry analysis of S100A8 and S100A9 expression in CD11b⁺CD14⁺ cells from a patient with non-small cell lung cancer (NSCLC) (CA) and normal subject (NC). Gray lines, IgG control; black lines, S100A8 or S100A9 staining. (B) Column bar graph analysis of S100A8 and S100A9 expression determined by fluorescence-activated cell sorter scan. The data are the mean $\pm$ SEM and are presented as the percentage of positively stained cells (left panel) and mean fluorescence intensity (MFI, right panel). Open bars, normal CD11b⁺CD14⁺ cells; solid bars, NSCLC CD11b⁺CD14⁺ cells; $n \geq 4$; $*P < 0.05$. (C) Correlation of S100A9⁺ cells in CD11b⁺CD14⁺ cells with the T-cell suppression ability of CD11b⁺CD14⁺ cells from patients with NSCLC. $n = 8$; Spearman $r = -0.85$; $P = 0.01$. (D) Correlation of the MFI of S100A9 to T-cell suppression ability from patients with NSCLC. Spearman $r = -0.89$; $n = 6$; $*P = 0.033$.

CD14⁺ cells from patients with NSCLC. $n = 8$; Spearman $r = -0.85$; $P = 0.01$. (D) Correlation of the MFI of S100A9 to T-cell suppression ability from patients with NSCLC. Spearman $r = -0.89$; $n = 6$; $*P = 0.033$.

patient's performance status and response to platinum-based doublet chemotherapy. These cells expressed CD15⁺CD33⁺IL-4R α ⁺ and produced a number of cytokines, chemokines, and growth factors. The suppressive effect on T-cell proliferation was not

restricted to the CD14⁺HLA-DR^{-/low} phenotype and was mediated by iNOS, arginase 1, the IL-13/IL-4R α axis, and IL-10. For the first time in human cancer, this study demonstrated that the expression of S100A9 in CD11b⁺CD14⁺ cells was correlated

Figure 6. Clinical relevance of CD11b⁺CD14⁺S100A9⁺ in non-small cell lung cancer (NSCLC). (A) Relationship of the percentage of CD11b⁺CD14⁺S100A9⁺ and chemotherapy response. Partial response (PR), stable disease (SD), progressive disease (PD), n = 9, 10, and 5, respectively. PR versus PD and SD versus PD, both $P < 0.05$. (B) Correlation of the percentage of CD11b⁺CD14⁺S100A9⁺ cells in the peripheral blood mononuclear cells (PBMCs) with progression-free survival after platinum-based doublet chemotherapy. Spearman $r = -0.83$; n = 24; $*P < 0.0001$. (C) Correlation of the percentage of CD11b⁺CD14⁺ cells in the PBMCs with progression-free survival (PFS) after platinum-based doublet chemotherapy. Spearman $r = -0.58$; n = 24; $*P = 0.001$. (D) Kaplan-Meier curve of PFS according to the median percentage of CD11b⁺CD14⁺S100A9⁺. Dark line, CD11b⁺CD14⁺S100A9⁺ $\leq 20\%$ in PBMCs; dashed gray line, CD11b⁺CD14⁺S100A9⁺ greater than 20%. Median survival 9.2 versus 3 months; hazard ratio (HR), 0.06; 95% confidence interval (CI), 0.02–0.23; log-rank test $P < 0.0001$. (E) Kaplan-Meier curve of PFS according to the median percentage of CD11b⁺CD14⁺. Dark line, CD11b⁺CD14⁺ $< 19.1\%$ in PBMC; dashed gray line, CD11b⁺CD14⁺ $> 19.1\%$ in PBMC. Median survival 9 versus 2.9 months; hazard ratio, 0.30; 95% confidence interval, 0.10–0.88; log-rank test $P = 0.03$. (F) Representative images of migrated carboxy-fluorescein diacetate succinimidyl ester (CFSE)-labeled CD14⁺ cells that were photographed with fluorescence microscopy after overnight culture in the presence of A549 cells in the bottom chamber in migration assays. S100A9 or anti-RAGE blocking antibody or IgG control was added in the CD14⁺ cell-seeded upper chamber. (G) Column bar graph analysis of the quantification of migrated cells as in (F) from four independent experiments. The data are presented as the mean $\pm$ SEM of cell numbers/field; $*P < 0.05$. (H) MTT assay analysis of A549 cells viability in response to cisplatin (0, 50, 100 mM) in the presence or absence of coculture with NSCLC CD14⁺ cells. The data are presented as the mean $\pm$ SEM of the percentage of cell viability relative to the corresponding control cells without cisplatin treatment. n = 3; $***P < 0.001$ compared with the corresponding CD14⁺ cells controls at each cisplatin concentration. CA = patients with NSCLC; NC = healthy donors.

with their capacity as MDSCs and clinical response to chemotherapy.

In the presence of immunosuppression, the immune mechanisms facilitating chemotherapeutic response, such as immunogenic cell death, are dampened (23). Although we previously reported the expansion of CD11b⁺CD14⁺ granulocytic MDSCs in patients with NSCLC (11), we did not observe an association of these cells with clinical relevance (e.g., performance status or treatment response to chemotherapy). Although CD11b⁺CD14⁺ subpopulation of MDSCs has an inhibitory ability comparable with the CD11b⁺CD14⁺ cells, it is composed of a small portion of suppressor cells that may explain the obscure clinical relevance.

In contrast, the amount of CD11b⁺CD14⁺ MDSCs is correlated with performance status and response to chemotherapy. Removal of CD14⁺ cells almost completely restores the inhibition of CD8⁺ T cells in PBMCs. These data suggest that of the general population of MDSCs, monocytic MDSCs are the cells that possess important clinical relevance in treatment-naïve patients with NSCLC. These CD11b⁺CD14⁺ cells are not restricted to the CD14⁺HLA-DR^{low} MDSCs, as shown in metastatic melanoma and hepatocellular carcinoma (15–17), because enriched CD14⁺HLA-DR^{low} cells showed a lower ability to inhibit T-cell proliferation than total CD14⁺ cells.

In contrast to patients with NSCLC, the CD11b⁺CD14⁺ cells of healthy subjects did not suppress T cells. The higher expression levels of iNOS and arginase 1 in the CD11b⁺CD14⁺ cells of patients with NSCLC may explain the difference. The up-regulation of iNOS and arginase 1 results in L-arginine deprivation and NO production, which in turn leads to the suppression of T-cell function and proliferation (24, 25). IL-4R α is functionally required for CD14⁺ MDSCs in mice (9) and has been suggested to be a marker of MDSCs in patients with colon cancer (12). In this study, we showed that neutralizing the antibody against IL-4R α reversed the inhibitory effect of NSCLC CD11b⁺CD14⁺ cells. IL-4R α activated by IL-13 potentiates the immunosuppressive effect of MDSCs by up-regulating arginase 1 (26). However, IL-4R α ⁺ cells isolated from healthy subjects do not suppress T cells. In addition, the expression level of IL-4R α in the CD14⁺IL-4R α ⁺ cells of patients with NSCLC was similar to the level in the healthy subjects. Taken together, the functional difference in CD14⁺ cells between patients with NSCLC and healthy subjects is not attributed to the expression of IL-4R α . Indeed, exogenous IL-13 transforms CD11b⁺CD14⁺ cells of healthy subjects into functionally active MDSCs (26). As such, the IL-13 produced by CD11b⁺CD14⁺ cells contributes to the IL-4R α -mediated effect. Although STAT3-dependent

oxidative stress is important for MDSCs in patients with metastatic melanoma (15), this pathway is not required for CD11b⁺CD14⁺ MDSCs in patients with NSCLC, indicating that the CD11b⁺CD14⁺ MDSCs in the present study are unique to the HLA-DR^{low} phenotype in patients with melanoma.

The results in this study showed that the pharmacologic inhibitors AG and NOHA and neutralizing antibodies to IL-10 or IL-4R α individually and completely reversed the inhibitory effects of CD14⁺ MDSCs on T-cell proliferation. By inhibiting the phosphorylation of the IL-2 receptor and decreasing IL-2 mRNA stability, iNOS suppressed T-cell proliferation. Arginase 1 had a similar effect by CD3 ζ down-regulation. Arginase 1 and iNOS also cooperatively suppressed T-cell proliferation (13). Thus, inhibition of either one could restore T-cell suppression. IL-10 is important and not redundant to the suppressive function of MDSCs (27). IL-10 has been shown to inhibit T-cell proliferation by inhibiting CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding (28). This inhibition seems to occur only when T cells are stimulated by low numbers of triggered T-cell receptors and is therefore CD28 costimulation-dependent. It is likely that significant inhibition of T cells by CD14⁺ MDSCs requires TCR (CD3 ζ) and CD28 to be simultaneously inhibited. In a murine model of graft-versus-host disease, IL-13 up-regulated arginase 1 expression and induced potent MDSCs, which displayed a suppressive function that was arginase 1-dependent (26). Our preliminary data also showed a trend of IL-10-dependent up-regulation of arginase 1 (data not shown). Thus, the IL-10 and IL-13/IL4R α pathways seem to converge on arginase 1, which suppresses T cells in cooperation with iNOS. Taken together, in CD14⁺ MDSCs, IL-10, IL13/IL4R α , iNOS, and arginase 1 seem to work in concert to inhibit T-cell activation. This is supported by the results of the current study. However, the precise mechanism of this inhibition requires further study.

Proinflammatory mediators, such as IL-1 β , IL-6, and prostaglandin E₂, induce the accumulation of MDSCs in tumor-bearing individuals (4). However, in a mouse model, MDSCs also accumulate in the absence of overtly elevated IL-1 β , IL-6, and prostaglandin E₂ levels. S100 proteins have been reported to sustain the accumulation of MDSC in mice through autocrine loop activation (20). In this study, we showed that the CD11b⁺CD14⁺ cells of patients with NSCLC expressed high levels of S100A8/A9 compared with the levels in healthy subjects. It was shown that the expression of S100A9 was highly correlated with the ability to suppress T-cell proliferation by CD11b⁺CD14⁺ cells. However, S100A9 does not seem to

play a direct role in suppressing T cells, because the RAGE blocking antibody failed to reverse CD14⁺ MDSC-induced T-cell suppression. These data are consistent with a previous report (20) and explain why the STAT3 inhibitor AG490, which was expected to down-regulate S100A9, had no effect on T-cell proliferation. Importantly, the expression of S100A9 protein was also correlated with clinical response and PFS for platinum-based chemotherapy. The S100A9 protein interacts with the TLR4 receptor or RAGE to activate the mitogen-activated protein kinase and nuclear factor- κ B pathways (29, 30), thereby activating specific genes that regulate MDSC recruitment, angiogenesis, tumor growth, and metastasis. *In vitro* studies confirmed the functional role of S100A9 in mediating the migration of CD14⁺ MDSCs to the tumor site. This study also demonstrated that when NSCLC CD14⁺ MDSCs were cocultured with A549 cells, tumor cells were protected from the cytotoxic effect of cisplatin. That is consistent with our previous report that tumor-associated macrophages are associated with poor response to EGFR-TKI therapy in patients with NSCLC (31). However, a specific neutralizing antibody for RAGE seems to only marginally reverse the protective effect of NSCLC MDSCs (see Figure E10). Therefore, the major function of CD14⁺ MDSC-derived S100A9 is to mediate the migration of MDSCs, at least to the tumor site. There may be other factors in addition to S100A9 that are implicated in the protective effect of CD14⁺ MDSCs on cisplatin-induced cytotoxicity. Thus, S100A9 proteins may direct the migration of CD11b⁺CD14⁺ MDSCs toward tumor cells to protect tumor cells from the cytotoxic effect of chemotherapy.

CD11b⁺CD14⁺ cells in patients with NSCLC secrete higher levels of cytokines, including TNF- α , IL-8, IL-10, and IL-13. There is increasing evidence that host factors, such as weight loss, poor performance status, and the host systemic inflammatory response, are linked in advanced cancer; the inflammatory response acts as an important tumor-stage-independent predictor of outcome (32). Inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, contribute to the development of cachexia in patients with cancer (33), and TNF- α and IL-8 induce tumorigenicity, angiogenesis, cancer metastasis, and anticancer treatment resistance (34–36). In this study, we found that the CD11b⁺CD14⁺ cells from patients with NSCLC secreted more HGF than those from healthy subjects. A recent study showed that patients with multiple myeloma with lower serum HGF concentration have a better response to chemotherapy (37). Furthermore, tumor-associated macrophages, which may be derived from CD11b⁺CD14⁺ monocytes, with an up-regulation of HGF mRNA expression were correlated with poor prognosis in patients with lung cancer (38). Therefore, inflammatory mediators and growth factors released from CD11b⁺CD14⁺ MDSCs may also be a link to poor performance status and clinical response.

In conclusion, CD11b⁺CD14⁺ monocytic MDSCs that express high S100A9 are the most clinically relevant MDSCs for patients with NSCLC. These MDSCs suppress T-cell activation through the common iNOS/arginase mechanisms and through mechanisms unique to this cell population (e.g., the IL-13/IL-4R α axis and IL-10). These MDSCs are Th2-skewing inflammatory monocytes that may be related to clinical conditions and treatment response. S100A9 can be used as a marker for these MDSCs in clinical surveillance, at least for NSCLC. Whether patients benefit from modulating these cells and how to achieve this modulation warrants further investigation.

Author disclosures are available with the text of this article at www.atsjournals.org.

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