

Immunosuppressive CD11b⁺Ly6C^{hi} monocytes in pristane-induced lupus mouse model

Huijuan Ma,^{*} Suigui Wan,^{*,1} and Chang-Qing Xia^{*,†,2}

^{*}Department of Hematology, Xuanwu Hospital, Capital Medical University, Beijing, China; and [†]Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, Florida, USA

RECEIVED APRIL 16, 2015; REVISED OCTOBER 20, 2015; ACCEPTED NOVEMBER 16, 2015. DOI: 10.1189/jlb.3A0415-158R

ABSTRACT

Myeloid-derived suppressor cells with immunosuppressive functions have been described to be associated with one of the mechanisms by which malignant tumors escape immune surveillance. However, little is known about the role of myeloid-derived suppressor cells in autoimmunity. In the current study, when we attempted to characterize the peritoneal cells in pristane-induced lupus model, as reported previously, we observed that there were markedly increased CD11b⁺Ly6C^{hi} monocytes. Surprisingly, this type of monocytes was almost phenotypically identical to the reported monocytic myeloid-derived suppressor cells. Further analysis on how these CD11b⁺Ly6C^{hi} cells affected T cell response showed that they strongly suppressed T cell proliferation *in vitro* in a manner dependent on cell–cell contact, NO, and PGE₂. In addition, we found that CD11b⁺Ly6C^{hi} monocytes inhibited Th1 differentiation but enhanced development of forkhead box p3⁺CD4⁺ regulatory T cells. Consistent with the *in vitro* experimental results, the *in vivo* adoptive cell transfer study showed that infusion of pristane-treated syngeneic CD11b⁺Ly6C^{hi} monocytes significantly suppressed the production of anti-keyhole limpet hemocyanin antibodies induced by keyhole limpet hemocyanin immunization. In addition, we found that CD11b⁺Ly6C^{hi} monocytes were also increased significantly in spleen and peripheral blood and showed immunosuppressive characteristics similar to their peritoneal counterparts. Our findings indicate that CD11b⁺Ly6C^{hi} monocytes in a pristane-induced lupus mouse model are monocytic myeloid-derived suppressor cells instead of inflammatory monocytes, as demonstrated previously. To our knowledge, this is the first to describe myeloid-derived suppressor cells in a pristane-induced lupus mouse model, which may lead to

a better understanding of the role of CD11b⁺Ly6C^{hi} monocytes in this specific pristane-induced lupus model. *J. Leukoc. Biol.* **99**: 1121–1129; 2016.

Introduction

SLE is a chronic inflammatory autoimmune disease. One of the major characteristics of SLE is the overproduction of autoantibodies against nuclear materials [1, 2]. The mechanisms underlying SLE are complex and include genetic, environmental factors and abnormalities of the innate and adaptive immune systems, all of which contribute to the initiation, maintenance, and progression of the disease [1, 2]. Recent experimental and clinical studies have placed new emphasis on the role of innate immune system in SLE [3–5].

Accumulating evidence suggests that adaptive immunity is initiated, programmed, and constantly regulated by innate immune cells [3–7]. In recent years, the MDSCs with immunosuppressive functions have been described in many pathophysiological conditions, such as cancer, chronic infection, autoimmune disorders, transplantation, sepsis, and trauma [8–13]. In mice, the heterogeneous populations of CD11b⁺Gr-1⁺ cells are known as MDSCs, which include immature monocytes/macrophages, dendritic cells, and granulocytes [14–16]. Generally, they can be classified monocytic MDSCs (CD11b⁺Ly6C^{hi}Ly6G^{low}) with monocyte-like morphology and granulocytic MDSCs (CD11b⁺Ly6C^{hi}Ly6G^{low}) with granulocyte-like morphology [17–19]. Although a variety of different mechanisms is involved in the immunosuppressive function of MDSCs, the predominant factors are iNOS and Arg1 [8, 20]. In addition, ROS, PGE₂, IDO, COX-2, IL-10, and tumor-derived factors, such as TGF- β , are also very important for the suppression [21–25]. Moreover, studies have also proven that direct cell–cell contact is required for the immunosuppressive functions of MDSCs [8, 11, 26], which have been identified in several mouse models of autoimmune diseases, such as EAE,

Abbreviations: APC = allophycocyanin, Arg1 = arginase 1, COX-2 = cyclooxygenase 2, EAE = experimental autoimmune encephalomyelitis, EIA = enzyme immunoassay, Foxp3 = forkhead box p3, KLH = keyhole limpet hemocyanin, MDSC = myeloid suppressor cell, ROS = reactive oxygen species, SLE = systemic lupus erythematosus, SMT = S-methylisothiourea, T_{reg} = regulatory T cell

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

1. Correspondence: Dept. of Hematology, Xuanwu Hospital, Capital Medical University, Beijing 100053, China. E-mail: wansuigui@hotmail.com
2. Correspondence: Dept. of Hematology, Xuanwu Hospital, Capital Medical University, Beijing 100053, China. E-mail: cqx65@yahoo.com

inflammatory bowel disease, rheumatoid arthritis, Type 1 diabetes, and uveoretinitis [11, 27–31]. However, it remains unclear with regard to their real roles in autoimmunity.

The pristane-induced lupus model, characterized by the development of high levels of lupus-specific autoantibodies and glomerulonephritis, has been used extensively to investigate the cellular mechanisms of SLE [32, 33]. It has been reported that CD11b⁺Ly6C^{hi} monocytes were responsible for increased type I IFN production, which was critically required for the pathogenesis of SLE [32–34]. In our current work, with the use of this model, we found that CD11b⁺Ly6C^{hi} monocytes in pristane-treated lupus mice were highly suppressive for the proliferation of CD4⁺ and CD8⁺ T cells through the production of NO and PGE2, as well as in a cell–cell contact fashion. In addition, Ly6C^{hi} monocytes significantly induced T_{regs} and suppressed Th1 cells. Moreover, adoptive transfer of purified CD11b⁺Ly6C^{hi} monocytes significantly suppressed the production of anti-KLH antibodies induced by KLH immunization. To our knowledge, this is the first to report MDSCs in a pristane-induced lupus model.

MATERIALS AND METHODS

Mice

Four-week-old female BALB/c mice were purchased from the Charles River Animal Facility (Beijing, China) and housed in a specific pathogen-free facility. At 8–10 wk of age, 10–15 mice/group received a single intraperitoneal injection of 0.5 ml pristane (Sigma-Aldrich, St. Louis, MO, USA) or PBS. Peritoneal cells, spleen, and blood were harvested 2 wk later. This study was approved by the Institutional Animal Care and Use Committee of Xuanwu Hospital, Capital Medical University (Beijing, China).

Antibodies and reagents

Fluorochrome-conjugated mouse mAb, including APC-CD11b (clone M1/70) and FITC-Ly6C (clone HK1.4) were purchased from BioLegend (San Diego, CA, USA). PerCP-CD4 (clone RM45) and PE-CD8 (clone 53-6.7) were from BD Biosciences (San Diego, CA, USA). PE-IFN- γ (clone AN.18.17.24), APC-IL-10 (clone JES5-16E3), and APC-Foxp3 (clone 3G3) or mouse IgG1 isotype control were purchased from Miltenyi Biotec (Auburn, CA, USA). The Foxp3 staining kit was from eBioscience (San Diego, CA, USA). CD11b, Ly6C, CD4, and CD8 were used for extracellular staining. IFN- γ , IL-10, and Foxp3 were used for intracellular staining. SMT (a selective inhibitor of iNOS) and indomethacin (a nonselective inhibitor of COX), were obtained from Sigma-Aldrich. Anti-KLH antibody kit was purchased from Life Diagnostics (West Chester, PA, USA).

Flow cytometric analysis

The peritoneal cavity was lavaged with 1 ml cold, sterile PBS, and the intraperitoneal fluid was harvested. Peritoneal cells were collected by centrifugation at 450 g for 10 min. For cell surface marker staining, peritoneal cells, splenocytes, and mononuclear cells from peripheral blood were prepared as single-cell suspensions. For intracellular cytokine staining, cells were stimulated with PMA (50 ng/ml) and ionomycin (1 μ g/ml) for 6 h. Brefeldin A (BioLegend) was added for the last 4 h of culture. After surface staining with PerCP-conjugated anti-CD4 mAb for 20 min at 4°C, cells were fixed, permeabilized, and stained with APC-conjugated anti-IL-10 or PE-conjugated anti-IFN- γ mAb for 30 min at 4°C. Intracellular staining for Foxp3 was performed, according to the manufacturer's instructions. Data acquisition was performed on a flow cytometer (FACSCanto II; BD Biosciences) and analyzed with FCS Express 4 (De Novo Software, ONT, Canada). For cell sorting experiments, peritoneal cells from pristane-treated mice were stained with anti-CD11b and anti-Ly6C and sorted using a FACS Aria (BD Biosciences) into CD11b⁺Ly6C^{hi} and CD11b⁺Ly6C^{mid} populations. In addition, splenocytes were stained with anti-CD11b and anti-Ly6C and sorted into CD11b⁺Ly6C^{hi} cells. The purity of these sorted populations was typically >95% (Supplemental Fig. 1).

Wright-Giemsa staining

For morphologic analysis, 2×10^5 sorted cells were cytopspun onto glass slides, air dried for 30 min, and stained using the Hema3 staining kit (Thermo Fisher Scientific, Waltham, MA, USA).

In vitro immunosuppression assay

RPMI 1640 (Invitrogen, Carlsbad, CA, USA), supplemented with 10% FBS (Invitrogen), 100 μ g/ml streptomycin, and 100 U/ml penicillin (complete medium), was used for in vitro coculture. Autologous splenocytes (5×10^5 cells/well) were labeled with CFSE, following the instructions from the manufacturer (Invitrogen), and were cultured alone or cocultured with sorted myeloid cell populations at different ratios (as indicated in the figures) in the presence of soluble anti-CD3 mAb (1 μ g/ml; BD Biosciences) in a 96-well, round-bottom plate for 96 h. Proliferation of T cells was determined based on CFSE dilution by flow cytometry.

Adoptive cell transfer and the measurement of KLH immunization-induced anti-KLH antibody

CD11b⁺Ly6C^{hi} cells were sorted from the peritoneal cells, 2 wk after pristane injection by flow cytometry. Then, naïve BALB/c female mice of the experimental group received intravenous injection of isolated CD11b⁺Ly6C^{hi} cells (5×10^6 cells/mouse), once/wk for 2 wk. The control group received the same volume of PBS. At the first transfusion, both groups were immunized with exogenous antigen KLH (20 μ g/mouse; Thermo Fisher Scientific, Rockford, IL, USA), mixed with adjuvant Alum (Thermo Fisher Scientific) by intraperitoneal injection. Two weeks after the KLH immunization, plasma samples were prepared and assayed for anti-KLH antibody using the Mouse Anti-KLH IgG ELISA kit (Life Diagnostics).

IL-10, TGF- β 1 detection, and NO and PGE2 measurement

Supernatants were collected, and the level of IL-10 was examined using the ELISA kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions, and the level of TGF- β 1 was measured using the Luminex TGF- β Bead Kit (EMD Millipore, Billerica, MA, USA). In addition, NO and PGE2 were measured by Griess reagent and EIA (Cayman Chemicals, Ann Arbor, MI, USA), respectively.

Transwell assays

To test contact dependency of MDSC-mediated T cell suppression, sorted CD11b⁺Ly6C^{hi} monocytes and fresh autologous, CFSE-labeled splenocytes were cocultured at the 1:2 ratio in the presence of anti-CD3 stimulation for 96 h, as described above. Transwell plates (0.4 μ m pores; Costar, Corning, NY, USA) were used for these studies, with splenocytes (5×10^5 cells/well) cultured in plate wells and monocytes (2.5×10^5 cells/well) cultured in Transwell inserts in complete medium in the total volume of 200 μ l to prevent direct contact between splenocytes and monocytes.

Statistical analysis

Data are presented as means \pm SEM. Statistical analysis was performed with the GraphPad Prism 4 package (GraphPad Software, San Diego, CA, USA). Groups were compared using a 2-tailed unpaired Student's *t* test. Dunnett's tests were used when multiple comparisons were performed in the same experiment. *P* < 0.05 was considered significant.

RESULTS

Pristane induces the accumulation of Ly6C^{hi} monocytes

To identify the cell types in the peritoneal exudates, 2 wk after pristane treatment, we use these 2 markers, CD11b and Ly6C, to distinguish the myeloid-derived cell populations. The total number of peritoneal cells in pristane-treated mice was much higher than that in PBS-treated mice (Fig. 1A). In PBS-treated

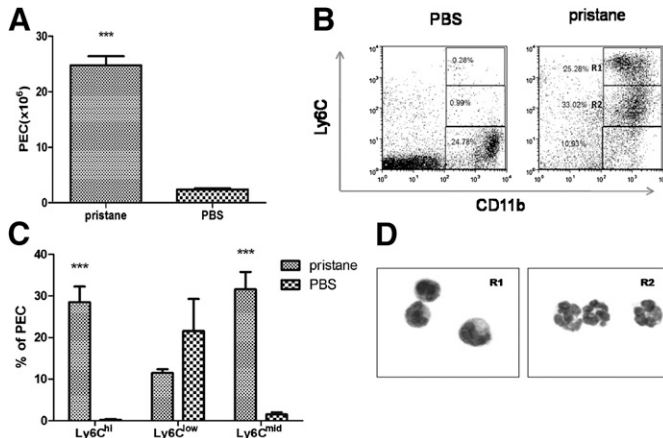


Figure 1. Pristane induces accumulation of Ly6C^{hi} monocytes. Two weeks after pristane injection, peritoneal cells were collected from the pristane and PBS groups and analyzed by flow cytometry. (A) Total peritoneal exudate cell (PEC) count. (B) Analysis of PEC by flow cytometry. Representative percentages of CD11b⁺Ly6C^{hi} immature monocytes, CD11b⁺Ly6C^{mid} neutrophils, and CD11b⁺Ly6C^{low} mature monocytes/macrophages were assessed. (C) Quantification of peritoneal cell populations. (D) Morphologic analysis of peritoneal CD11b⁺Ly6C^{hi} monocytes (R1) and CD11b⁺Ly6C^{mid} neutrophils (R2) from pristane-treated mice; $n = 10$ –15 mice/group. Data are representative of 3 independent experiments. *** $P < 0.001$ vs. the corresponding PBS group.

mice, peritoneal cells mainly consist of B1 cells (CD11b^{low/mid}Ly6C⁺) and mature monocytes/macrophages (CD11b^{hi}Ly6C⁺; Fig. 1B). Interestingly, these 2 cell types disappeared 2 wk after pristane treatment, and the other 2 types of cells were predominant, which were CD11b⁺Ly6C^{hi} immature monocytes and CD11b⁺Ly6C^{mid} neutrophils (Fig. 1B and C) [32, 33]. Consistent with the immunophenotypic characteristics, the morphologic features by Wright-Giemsa staining showed that CD11b⁺Ly6C^{hi} monocytes were exclusively large mononuclear cells (Fig. 1D), whereas CD11b⁺Ly6C^{mid} neutrophils had ring-shaped nuclei (Fig. 1D).

Ly6C^{hi} monocytes potently suppress proliferation of T cells

Although CD11b⁺Ly6C^{hi} monocytes have been demonstrated to be associated with the pathogenesis of the pristane-induced mouse lupus model through secreting type I IFN [32–34], it is yet unknown how CD11b⁺Ly6C^{hi} monocytes affect T cells. In the following experiments, we sought to study how CD11b⁺Ly6C^{hi} monocytes interfered with the proliferation of CD4⁺ and CD8⁺ T cells in vitro. We purified CD11b⁺Ly6C^{hi} and CD11b⁺Ly6C^{mid} cells through FACS sorting and then cocultured them with autologous splenocytes at different ratios, as indicated in the Fig. 2. We found that CD11b⁺Ly6C^{hi} monocytes potently inhibited T cell proliferation, whereas CD11b⁺Ly6C^{mid} neutrophils failed to suppress T cells (Fig. 2A and B). Specifically, when splenocytes were cultured with anti-CD3 antibodies alone, the percentage of proliferating CD4⁺ T cells was $76.3 \pm 2.2\%$ in

total CD4⁺ T cells, whereas the percentages reduced to $52.5 \pm 5.4\%$ and $30.8 \pm 4.6\%$, respectively, for 1:8 and 1:4 cocultures (monocytes/splenocytes), and it was only $11.1 \pm 3.5\%$ for a 1:2 ratio coculture (Fig. 2C and E). Likewise, strong, suppressive effects of CD11b⁺Ly6C^{hi} monocytes on CD8⁺ T cells were also observed. The percentage of proliferating CD8⁺ T cells was $84.2 \pm 3.3\%$ when splenocytes were cultured with anti-CD3 antibodies alone and dropped to $56.9 \pm 1.3\%$, $30.7 \pm 0.7\%$, and $5.0 \pm 1.0\%$, respectively, for 1:8, 1:4, and 1:2 ratio cocultures (Fig. 2D and F). These data show that Ly6C^{hi} monocytes suppress T cell proliferation in vitro in a dose-dependent manner.

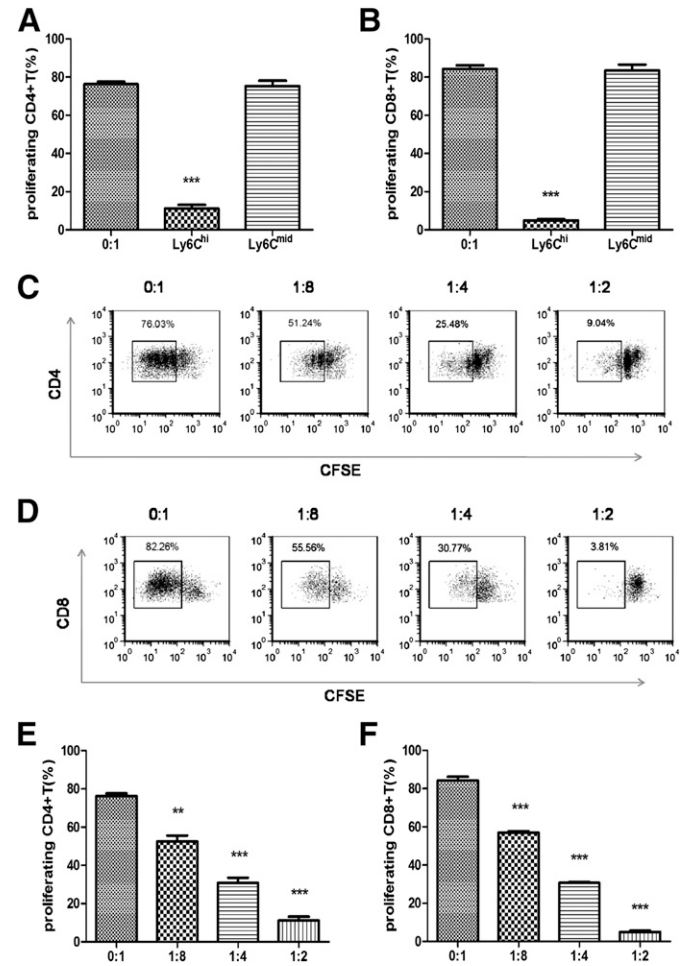


Figure 2. Ly6C^{hi} monocytes strongly suppress the proliferation of CD4⁺ and CD8⁺ T cells. CD11b⁺Ly6C^{hi} monocytes and CD11b⁺Ly6C^{mid} neutrophils were sorted by flow cytometry and cocultured with CFSE-labeled splenocytes (5×10^5 cells/well) in complete medium in the presence of anti-CD3. Proliferation assays were performed after 96 h coculture. (A and B) The coculture was performed at a 0:1 or 1:2 ratio (monocytes/splenocytes). The proliferation assay showed that only CD11b⁺Ly6C^{hi} monocytes could suppress the proliferation of CD4⁺ and CD8⁺ T cells. (C and D) CD11b⁺Ly6C^{hi} monocytes were cocultured with splenocytes at increasing ratios from 0:1 to 1:2, and cell proliferation was examined. Representative percentages of proliferating CD4⁺ and CD8⁺ T cells at different ratios. (E and F) Quantification of proliferation of CD4⁺ and CD8⁺ T cells at different ratios; $n = 6$ –8 mice/group. Data are representative of 3 independent experiments. ** $P < 0.01$ and *** $P < 0.001$ vs. the splenocyte-only group (0:1 group).

Adoptive transfer of pristane-induced CD11b⁺Ly6C^{hi} monocytes significantly suppresses the production of anti-KLH antibodies induced by KLH immunization

To verify whether this type of CD11b⁺Ly6C^{hi} monocyte has an immunosuppressive function in vivo, we used cell-adoptive transfer to study whether the transfer of purified CD11b⁺Ly6C^{hi} monocytes was able to suppress anti-KLH antibody development induced by KLH immunization. The results demonstrated that adoptive transfer of pristane-induced CD11b⁺Ly6C^{hi} monocytes significantly suppressed the production of anti-KLH antibodies (Fig. 3).

CD11b⁺Ly6C^{hi} monocyte-mediated T cell suppression depends on their NO production

To investigate the possible role of iNOS in mediating the suppressive function of CD11b⁺Ly6C^{hi} monocytes, we used its inhibitor SMT in the cocultures. The data showed that SMT treatment almost fully restored CD4⁺ and CD8⁺ T cell proliferation (Fig. 4A and B). To determine whether the inhibitor works in a concentration-dependent manner, we used a series of concentrations of iNOS inhibitor (5, 25, and 125 μ M) in the coculture system. The data showed that the iNOS inhibitor played a role in a concentration-dependent manner (Fig. 4C). Furthermore, we measured the levels of NO in the culture supernatants and found that SMT treatment could completely blocked NO production (Fig. 4D). These data suggest that NO produced by CD11b⁺Ly6C^{hi} monocytes plays a critical role in the immunosuppressive function.

PGE2 produced by CD11b⁺Ly6C^{hi} monocytes suppresses T cell proliferation

Given that PGE2 was reported to be associated with the immunosuppressive function of MDSCs [11, 35], we studied the effect of PGE2 produced by CD11b⁺Ly6C^{hi} monocytes on T cell proliferation, with or without use of its inhibitor, indomethacin.

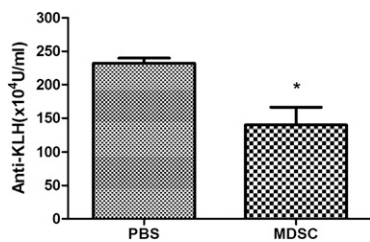


Figure 3. Adoptive transfer of purified CD11b⁺Ly6C^{hi} monocytes significantly suppresses the production of anti-KLH antibodies induced by KLH immunization. CD11b⁺Ly6C^{hi} monocytes were sorted from the peritoneal cells, 2 wk after pristane injection by flow cytometry. Then, naïve BALB/c mice of the MDSC group received the isolated CD11b⁺Ly6C^{hi} monocytes (5×10^6 cells/mouse) by intravenous injection, once/wk for 2 wk. The PBS group received the same volume of PBS. At the first transfusion, both groups were immunized with exogenous antigen KLH (20 μ g/mouse), mixed with adjuvant Alum by intraperitoneal injection. Two weeks after the KLH immunization, the anti-KLH antibody levels in the plasma were assayed using the ELISA kit; $n = 5$ mice/group. Data are representative of 3 independent experiments. * $P < 0.05$ vs. the corresponding PBS group.

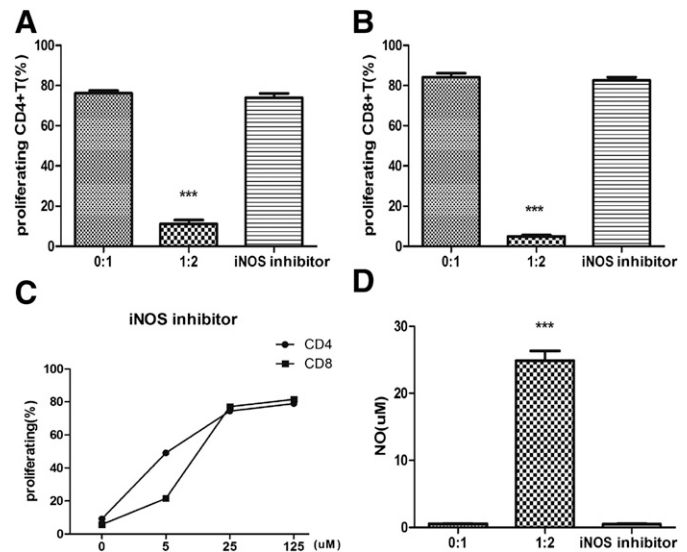


Figure 4. T cell suppression by Ly6C^{hi} monocytes depends on the NO production. The coculture was performed at a 0:1 or 1:2 ratio. (A and B) Splenocytes were cultured alone or cocultured with CD11b⁺Ly6C^{hi} monocytes isolated from pristane-treated mice. Cocultured cells were treated with or without the use of 125 μ M SMT (a selective inhibitor of iNOS). Proliferation was examined and showed the reversal of suppression by the iNOS inhibitor. (C) A series of concentrations of the iNOS inhibitor (5, 25, and 125 μ M) was added to the coculture system. Proliferation was examined and showed that the iNOS inhibitor worked in a concentration-dependent manner. (D) Supernatant was collected, and the level of NO was examined by Griess reagent; $n = 5$ –8 mice/group. Data are representative of 3 independent experiments. *** $P < 0.001$ vs. the splenocyte-only group (0:1 group).

The data showed that indomethacin contributed to a large proportion of recovery of CD4⁺ and CD8⁺ T cell proliferation (Fig. 5A and B) and exerted the effect also in a concentration-dependent manner (Fig. 5C). Moreover, indomethacin markedly suppressed PGE2 production in the supernatants (Fig. 5D).

CD11b⁺Ly6C^{hi} monocytes suppress T cells in a cell-cell contact manner

To test the contact dependency of Ly6C^{hi} monocyte-mediated T cell suppression, we separated splenocytes and CD11b⁺Ly6C^{hi} monocytes in the Transwell plate and found that Transwell coculture could completely abrogate the suppression (Fig. 6A and B). These data suggest that close cellular contact is required for the suppression. Furthermore, we compared the levels of NO and PGE2 in Transwell coculture with those produced in regular coculture and found that NO and PGE2 levels were decreased significantly in Transwell coculture (Fig. 6C and D).

CD11b⁺Ly6C^{hi} monocytes inhibit the Th1 response and enhance the generation of T_{regs}

To assess how CD11b⁺Ly6C^{hi} monocytes affect CD4⁺ T cell differentiation, we studied the cytokine-producing capability of CD4⁺ T cells, as well as the generation of Foxp3⁺CD4⁺ T_{regs} influenced by the coculture with CD11b⁺Ly6C^{hi} monocytes.

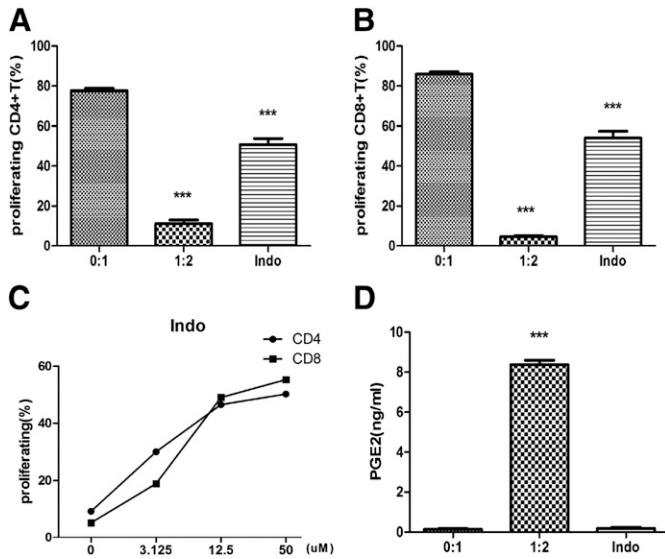


Figure 5. PGE2 produced by CD11b⁺Ly6C^{hi} monocytes suppresses T cell proliferation. The coculture was performed at a 0:1 or 1:2 ratio. (A and B) Splenocytes were cultured alone or cocultured with CD11b⁺Ly6C^{hi} monocytes isolated from pristane-treated mice. Cocultured cells were treated with or without the use of 50 μM indomethacin (Indo; a nonselective inhibitor of COX). Proliferation assay was performed and showed a large proportion of recovery of T cell proliferation by indomethacin. (C) A series of concentrations of indomethacin (3.125, 12.5, and 50 μM) was added to the coculture system. Proliferation was examined and showed that indomethacin worked in a concentration-dependent manner. (D) Supernatant was collected, and the level of PGE2 was examined by EIA; *n* = 5–8 mice/group. Data are representative of 3 independent experiments. ****P* < 0.001 vs. the splenocyte-only group (0:1 group).

We found that in the presence of CD11b⁺Ly6C^{hi} monocytes, IFN-γ-producing CD4⁺ T cells were significantly decreased and IL-10-producing CD4⁺ T cells increased (Fig. 7A and B). In addition, the coculture of T cells with CD11b⁺Ly6C^{hi} monocytes increased levels of Foxp3⁺ T cells (14.8 ± 1.2%), which were significantly higher than 8.5 ± 0.9% in the cultures without CD11b⁺Ly6C^{hi} monocytes (Fig. 7C), suggesting that CD11b⁺Ly6C^{hi} monocytes promoted T_{reg} differentiation. Meanwhile, we found that the levels of IL-10 in the supernatants were greatly elevated with the addition of Ly6C^{hi} monocytes (Fig. 7D) and so were the levels of TGF-β1 but to a lesser degree (Supplemental Fig. 2). Taken together, CD11b⁺Ly6C^{hi} monocytes suppress differentiation of Th1 but enhance generation of Foxp3⁺ and IL-10-producing T_{regs}.

CD11b⁺Ly6C^{hi} monocytes increase in the peripheral blood and spleen

To determine the changes of CD11b⁺Ly6C^{hi} monocytes in spleen and blood, induced by the treatment of pristane, we examined the frequency of CD11b⁺Ly6C^{hi} monocytes in the peripheral blood and spleen, 2 wk after the treatment. In PBS-treated mice, the percentages of CD11b⁺Ly6C^{hi} monocytes in blood and spleen were 0.24 ± 0.11% and 0.21 ± 0.06%, respectively, whereas in pristane-treated mice, the corresponding percentages were

increased significantly to 1.43 ± 0.51% and 0.63 ± 0.10%, respectively (Fig. 8A–C). Furthermore, similar to peritoneal CD11b⁺Ly6C^{hi} monocytes, we found that CD11b⁺Ly6C^{hi} monocytes from the spleen also greatly suppressed the proliferation of T cells (Fig. 8D).

DISCUSSION

In the pristane-induced lupus model, CD11b⁺Ly6C^{hi} monocytes and CD11b⁺Ly6C^{mid} neutrophils dominate the recruited cell populations in the peritoneal exudates, 2 wk after pristane peritoneal injection. This dramatic change would presumably be associated with the pathogenic process in the pristane-induced lupus mouse model. Indeed, it was reported that pristane-induced CD11b⁺Ly6C^{hi} monocytes secreted high levels of type I IFN, which was thought to be associated with the disease process in the pristane-induced lupus model [32–34]. However, our findings in the current study appear controversial to the results reported previously [32–34]. To our knowledge, this is the first to demonstrate that pristane-induced CD11b⁺Ly6C^{hi} monocytes are actually monocytic MDSCs showing a potent, suppressive effect on the proliferation of CD4⁺ and CD8⁺ T cells. In the MRL (Murphy Roths Large)-Fas^{lpr} mouse, CD11b⁺GR-1^{low} MDSCs with

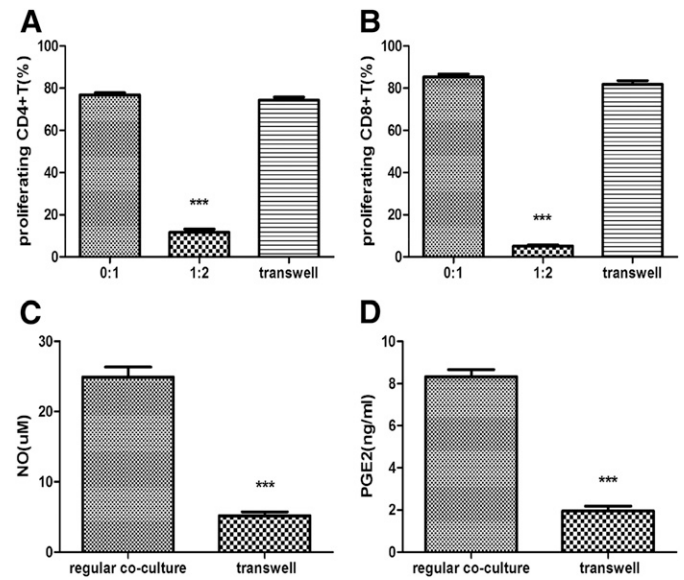


Figure 6. CD11b⁺Ly6C^{hi} monocytes suppress T cells in a cell-cell contact manner. (A and B) Splenocytes (5 × 10⁵ cells/well) and CD11b⁺Ly6C^{hi} monocytes (2.5 × 10⁵ cells/well) were cocultured in complete medium in the total volume of 200 μl at a 0:1 or 1:2 ratio. Transwell plates were used to test contact dependency of Ly6C^{hi} monocyte-mediated T cell suppression. Proliferation was examined and showed the reversal of suppression by Transwell coculture. (C) Supernatants were collected, and the level of NO was examined by Griess reagent and compared with that in regular cocultures. (D) Supernatants were collected, and the level of PGE2 was examined by EIA and compared with that in regular cocultures; *n* = 5–8 mice/group. Data are representative of 3 independent experiments. ****P* < 0.001 vs. the splenocyte-only group (0:1 group).

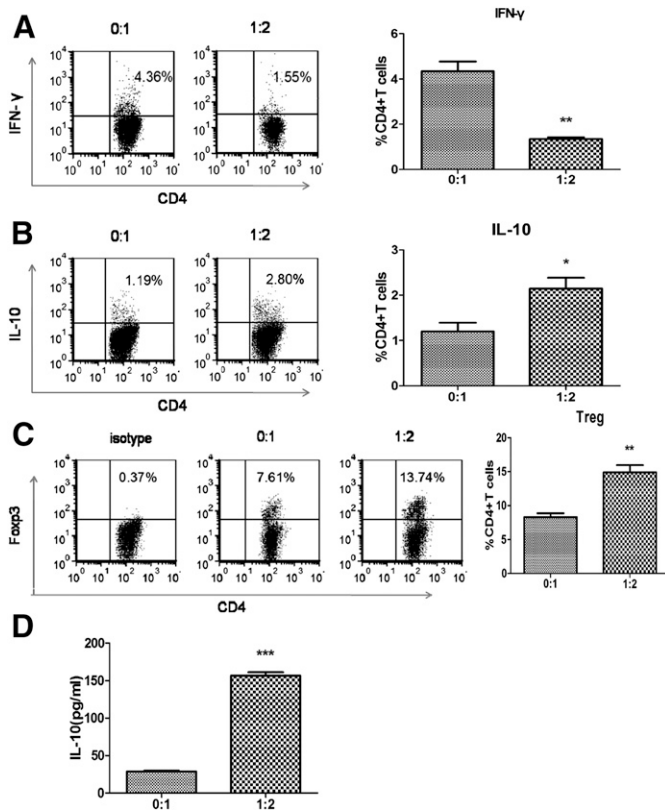


Figure 7. Ly6C^{hi} monocytes inhibit Th1 but promote induction of Treg_s. Splenocytes were cultured alone or cocultured with CD11b⁺Ly6C^{hi} monocytes at a 1:2 ratio in the presence of anti-CD3 (1 μg/ml) for 96 h. Data acquisition was performed on a flow cytometer. For each sample, at least 100,000 events were collected. (A) T cells cocultured with Ly6C^{hi} monocytes produced less IFN-γ. Representative intracellular staining plots and quantification of IFN-γ-producing CD4⁺ T cells were shown. (B) T cells cocultured with Ly6C^{hi} monocytes produced more IL-10. Representative intracellular staining plots and quantification of IL-10-producing CD4⁺ T cells were shown. (C) Ly6C^{hi} monocytes promoted generation of Foxp3⁺CD4⁺ T cells. Isotype control was used in the experiment. Representative intracellular staining plots and quantification of CD4⁺Foxp3⁺ cells in splenocytes were shown. (D) Supernatants were collected, and the levels of IL-10 were examined by ELISA; *n* = 5 mice/group. Data are representative of 3 independent experiments. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 vs. the splenocyte-only group (0:1 group).

monocytic morphology have been demonstrated to be able to suppress significantly CD4⁺ T cell proliferation in vitro and contribute to the autoimmune disorder [36]. In other models of SLE, there have not been reports about MDSCs.

MDSCs mainly consist of 2 populations: CD11b⁺Ly6C^{hi} Ly6G^{low} monocytes and CD11b⁺Ly6G^{hi}Ly6C^{low} neutrophils [8, 17, 19]. CD11b is a myeloid lineage differentiation antigen. Ly6C can be expressed on monocytes/macrophages, NK cells, endothelial cells, thymocytes, T cell subsets, and plasma cells [37]. The function of these molecules is not completely clear. It has been reported that bone marrow monocytes, representing earlier developmental stages, express a much higher level of Ly6C compared with blood

monocytes at steady state [38]. In line with this finding, we observed that the CD11b⁺Ly6C^{hi} population potently suppressed the T cell response. However, unlike the previous reports that CD11b⁺Ly6C^{mid} granulocytes were also immune suppressor cells [39], we found that CD11b⁺Ly6C^{mid} granulocytes lacked suppressive function. Thus, we focused our study on the characterization of immunosuppressive function of pristane-induced CD11b⁺Ly6C^{hi} monocytes.

Immunophenotypically, pristane-induced CD11b⁺Ly6C^{hi} monocytes are identical to the reported monocytic MDSCs [8, 27, 40]. In evaluating whether pristane-induced CD11b⁺Ly6C^{hi} monocytes are functionally the same as monocytic MDSCs, we found that the sorted CD11b⁺Ly6C^{hi} monocytes suppressed CD4⁺ and CD8⁺ T cell proliferation in

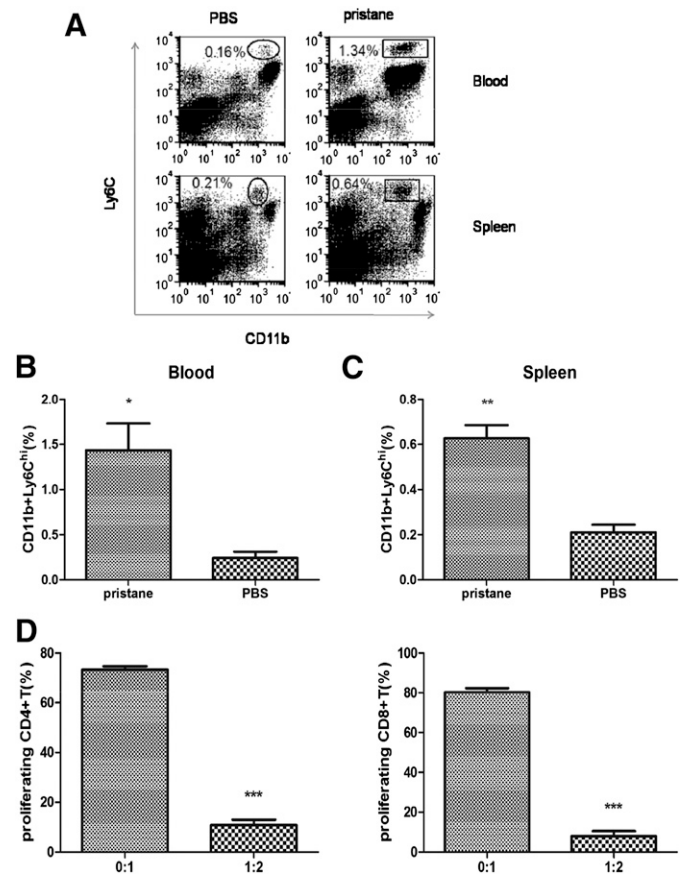


Figure 8. Characterization of Ly6C^{hi} monocytes in the peripheral blood and spleen, 2 wk after pristane injection. (A) Analysis of Ly6C^{hi} monocytes in blood and spleen by flow cytometry. The flow analysis was based on the gated total live cells. Representative percentages were exhibited. (B) Quantification of peripheral blood Ly6C^{hi} monocytes was shown. (C) Quantification of splenic Ly6C^{hi} monocytes was shown. (D) CD11b⁺Ly6C^{hi} monocytes from the spleen were sorted by flow cytometry and cocultured with CFSE-labeled splenocytes at a 0:1 or 1:2 ratio in the presence of anti-CD3 for 96 h. Quantification of proliferation of CD4⁺ and CD8⁺ T cells was shown, respectively; *n* = 7–10 mice/group. Data are representative of 3 independent experiments. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 vs. the corresponding PBS group.

a manner dependent on NO, PGE2, and cell–cell contact, which has been reported to be the property of MDSCs [8, 11, 18, 35]. Furthermore, our findings are very similar to the study by Zhu et al. [27] that CD11b⁺Ly6C^{hi}Ly6G^{low} MDSCs isolated from spleen following induction of EAE potentially inhibited the proliferation of CD4⁺ and CD8⁺ T cells *ex vivo*, which was mediated by iNOS activity. In addition, we demonstrated that pristane-induced CD11b⁺Ly6C^{hi} monocytes inhibited a Th1 response and enhanced generation of Foxp3⁺CD4⁺ as well as IL-10-producing T_{regs}. These findings are in line with the study by Kurmaeva et al. [11] that Ly6C^{hi} monocytes from colon lamina propria limited Th1 differentiation and induced T_{reg} generation. Importantly, our *in vivo* study had shown that adoptively transferred pristane-induced CD11b⁺Ly6C^{hi} monocytes significantly suppressed the production of anti-KLH antibodies induced by KLH immunization. All of the evidence described above suggests that Ly6C^{hi} monocytes, which have been reported to be inflammatory, may be an important population with regulatory function in restraining the immune response locally during an acute phase of lupus induction by injection of pristane. Moreover, the percentages of CD11b⁺Ly6C^{hi} monocytes in blood and spleen in pristane-induced mice are also increased significantly compared with those in PBS-treated mice, and the sorted CD11b⁺Ly6C^{hi} monocytes from the spleen had a potent, suppressive effect on the proliferation of T cells as well, suggesting that they might also be important immune regulators systemically during lupus induction.

There are some differences between MDSCs in tumor models and CD11b⁺Ly6C^{hi} monocytes in our lupus model. MDSCs from tumor models express iNOS and Arg1, and only inhibiting both enzymes can reverse the suppression [41, 42]. However, in our lupus model, T cell proliferation can be restored completely via the use of the iNOS inhibitor or mostly restored via the use of the PGE2 inhibitor. In our study, Transwell coculture could greatly reduce the levels of NO and PGE2 and completely restored proliferation of T cells, indicating that close cellular contact may be required for the release of NO and PGE2 by CD11b⁺Ly6C^{hi} monocytes upon receiving signaling from the cocultured T cells. These results are consistent with the findings by Kurmaeva et al. [11] that Ly6C^{hi} monocytes from colon lamina propria produced much more NO when cocultured with activated T cells than when cultured alone. A recent study by Grainger et al. [35] reported that Ly6C^{hi} monocytes recruited to the inflammatory sites during acute gastrointestinal inflammation limited activation of neutrophils via a PGE2-dependent mechanism, further supporting the critical role of PGE2 in the suppressive function of CD11b⁺Ly6C^{hi} monocytes. In our study, only the Ly6C^{hi} monocytic MDSC subset could potentially suppress proliferation of T cells *in vitro*. A review paper by Gabrilovich and Nagaraj [8] pointed out that the monocytic MDSC subset exhibited up-regulated expression of iNOS and increased levels of NO but little ROS production, whereas the granulocytic MDSC subset produced high levels of ROS but little NO production. These findings, along with our results in the current study, strongly support that NO but not ROS is the major immunosuppressive factor produced by monocytic

MDSCs. Other mechanisms, such as free radical production, are also implicated in T cell suppression in certain particular models [43].

It is widely believed that T_{regs} represent 1 important regulatory mechanism in restricting inflammation [44]. Huang et al. [45] reported that Gr-1⁺CD115⁺ MDSCs could induce the development of Foxp3⁺ CD4⁺ T_{regs} and mediate the inactivation of tumor-specific T cells *in vivo* in tumor-bearing mice. In addition, in Type 1 diabetes, expansion of MDSCs after anti-CD20 therapy was shown to induce T_{reg} differentiation in a TGF- β -dependent manner in nonobese diabetic mice [26]. The ability of MDSCs to induce proliferation/development of T_{regs} was confirmed recently in a human setting. A study in hepatocellular carcinoma patients [46] suggested that CD14⁺HLA-DR^{-/low} MDSCs isolated from PBMCs exerted their immunosuppressive function through the induction of IL-10-producing CD4⁺CD25⁺Foxp3⁺ T_{regs} when cocultured with autologous CD3/CD28-stimulated T cells. In accordance with these reports, our data show that CD11b⁺Ly6C^{hi} monocytes significantly enhance the generation of Foxp3⁺CD4⁺ T cells, which is consistent with the increased levels of TGF- β 1 in the cocultures with CD11b⁺Ly6C^{hi} monocytes (Supplemental Fig. 2), and in the meantime, restrain Th1 cells. These findings suggest that pristane-induced CD11b⁺Ly6C^{hi} monocytes may suppress the disease process of lupus, partly through induction of T_{regs} and suppression of Th1.

Controversial reports exist as to the role of MDSCs in the pathogenesis of autoimmune diseases. This might be a result of the discrepancy between *in vitro* and *in vivo* studies. The Mildner study [47] and the King study [48] demonstrated that CD11b⁺Ly6C^{hi} monocytes, accumulated in the CNS *in vivo*, served as pathogenic effectors and were associated with EAE pathogenesis. On the other hand, adoptively transferred MDSCs can limit autoimmune pathology, as observed in mouse models of Type 1 diabetes and inflammatory bowel disease [30, 49]. A recent study reported that deletion of CD24 in a lupus-like disease model led to the development of MDSCs and T_{regs} that augmented immune tolerance, accompanied with the alleviation of lupus-like renal pathology [50]. The *in vivo* experiment in our study demonstrated that adoptive transfer of pristane-induced CD11b⁺Ly6C^{hi} monocytes significantly suppressed the production of anti-KLH antibodies induced by KLH immunization, supporting their immunosuppressive function *in vivo*. The cellular interactions *in vivo* are much more complicated and cannot be recapitulated *in vitro*. Further studies are needed to address the role of CD11b⁺Ly6C^{hi} monocytes in the pristane-induced lupus model *in vivo*.

Taken together, based on our *in vitro* data, CD11b⁺Ly6C^{hi} monocytes, as a specific population of innate immune cells, are effective to suppress T cell proliferation, suggesting that they may play a protective role in chronic autoimmune inflammation in pristane-induced lupus, which is controversial to the literature [32]. Further studies to elucidate the real roles of this population as a potential negative regulator of inflammation in the autoimmune disease are required. The unraveling of the cellular and molecular requirements underlying their migration, differentiation, and maintenance and further characterization of their *in vivo* function

will help to reveal the role of CD11b⁺Ly6C^{hi} monocytes in lupus development, as well as identify molecules that can be targeted for developing therapeutic approaches.

AUTHORSHIP

H.M. performed the experiments, analyzed the data, and wrote the paper. S.W. designed and performed part of the experiments. C.-Q.X. conceived of the study, designed the experiments, and wrote the paper.

ACKNOWLEDGMENTS

This study was supported by Natural Science Foundation of China (81373192 and 81172854). The authors thank Mr. Yuezhong Zhao at the Department of Laboratory Animals, Capital Medical University, for the assistance in animal care.

DISCLOSURES

All authors declare that there are no conflicts of interest.

REFERENCES

1. Tsokos, G. C. (2011) Systemic lupus erythematosus. *N. Engl. J. Med.* **365**, 2110–2121.
2. Rahman, A., Isenberg, D. A. (2008) Systemic lupus erythematosus. *N. Engl. J. Med.* **358**, 929–939.
3. Llanos, C., Mackern-Oberti, J. P., Vega, F., Jacobelli, S. H., Kalgis, A. M. (2013) Tolerogenic dendritic cells as a therapy for treating lupus. *Clin. Immunol.* **148**, 237–245.
4. Dema, B., Charles, N. (2014) Advances in mechanisms of systemic lupus erythematosus. *Discov. Med.* **17**, 247–255.
5. Sharma, S., Campbell, A. M., Chan, J., Schattgen, S. A., Orlowski, G. M., Nayar, R., Huyler, A. H., Nündel, K., Mohan, C., Berg, L. J., Shlomchik, M. J., Marshak-Rothstein, A., Fitzgerald, K. A. (2015) Suppression of systemic autoimmunity by the innate immune adaptor STING. *Proc. Natl. Acad. Sci. USA* **112**, E710–E717.
6. Volz, T., Kaesler, S., Skabytska, Y., Biedermann, T. (2015) [The role of the innate immune system in atopic dermatitis]. *Hautarzt* **66**, 90–95.
7. Béland, S., Désy, O., Vallin, P., Basoni, C., De Serres, S. A. (2015) Innate immunity in solid organ transplantation: an update and therapeutic opportunities. *Expert Rev. Clin. Immunol.* **11**, 377–389.
8. Gabrilovich, D. I., Nagaraj, S. (2009) Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* **9**, 162–174.
9. Bunt, S. K., Sinha, P., Clements, V. K., Leips, J., Ostrand-Rosenberg, S. (2006) Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *J. Immunol.* **176**, 284–290.
10. Rabinovich, G. A., Gabrilovich, D., Sotomayor, E. M. (2007) Immunosuppressive strategies that are mediated by tumor cells. *Annu. Rev. Immunol.* **25**, 267–296.
11. Kurmaeva, E., Bhattacharya, D., Goodman, W., Omenetti, S., Merendino, A., Berney, S., Pizarro, T., Ostanin, D. V. (2014) Immunosuppressive monocytes: possible homeostatic mechanism to restrain chronic intestinal inflammation. *J. Leukoc. Biol.* **96**, 377–389.
12. Boros, P., Ochando, J. C., Chen, S. H., Bromberg, J. S. (2010) Myeloid-derived suppressor cells: natural regulators for transplant tolerance. *Hum. Immunol.* **71**, 1061–1066.
13. Cuenca, A. G., Delano, M. J., Kelly-Scumpia, K. M., Moreno, C., Scumpia, P. O., Laface, D. M., Heyworth, P. G., Efron, P. A., Moldawer, L. L. (2011) A paradoxical role for myeloid-derived suppressor cells in sepsis and trauma. *Mol. Med.* **17**, 281–292.
14. Bronte, V., Apolloni, E., Cabrelle, A., Ronca, R., Serafini, P., Zamboni, P., Restifo, N. P., Zanovello, P. (2000) Identification of a CD11b(+) / Gr-1(+) / CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. *Blood* **96**, 3838–3846.
15. Kusmartsev, S., Gabrilovich, D. I. (2006) Role of immature myeloid cells in mechanisms of immune evasion in cancer. *Cancer Immunol. Immunother.* **55**, 237–245.
16. Serafini, P., Borrello, I., Bronte, V. (2006) Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. *Semin. Cancer Biol.* **16**, 53–65.
17. Youn, J. I., Nagaraj, S., Collazo, M., Gabrilovich, D. I. (2008) Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J. Immunol.* **181**, 5791–5802.
18. Movahedi, K., Williams, M., Van den Bossche, J., Van den Bergh, R., Gysmans, C., Beschin, A., De Baetselier, P., Van Ginderachter, J. A. (2008) Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood* **111**, 4233–4244.
19. Ribechini, E., Greifengberg, V., Sandwick, S., Lutz, M. B. (2010) Subsets, expansion and activation of myeloid-derived suppressor cells. *Med. Microbiol. Immunol. (Berl.)* **199**, 273–281.
20. Bronte, V., Zanovello, P. (2005) Regulation of immune responses by L-arginine metabolism. *Nat. Rev. Immunol.* **5**, 641–654.
21. Sinha, P., Clements, V. K., Fulton, A. M., Ostrand-Rosenberg, S. (2007) Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res.* **67**, 4507–4513.
22. Li, H., Han, Y., Guo, Q., Zhang, M., Cao, X. (2009) Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. *J. Immunol.* **182**, 240–249.
23. Corzo, C. A., Cotter, M. J., Cheng, P., Cheng, F., Kusmartsev, S., Sotomayor, E., Padhya, T., McCaffrey, T. V., McCaffrey, J. C., Gabrilovich, D. I. (2009) Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J. Immunol.* **182**, 5693–5701.
24. Mellor, A. L., Munn, D. H. (2004) IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat. Rev. Immunol.* **4**, 762–774.
25. Sinha, P., Clements, V. K., Bunt, S. K., Albelda, S. M., Ostrand-Rosenberg, S. (2007) Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J. Immunol.* **179**, 977–983.
26. Hu, C., Du, W., Zhang, X., Wong, F. S., Wen, L. (2012) The role of Gr1+ cells after anti-CD20 treatment in type 1 diabetes in nonobese diabetic mice. *J. Immunol.* **188**, 294–301.
27. Zhu, B., Bando, Y., Xiao, S., Yang, K., Anderson, A. C., Kuchroo, V. K., Khoury, S. J. (2007) CD11b+Ly-6C(hi) suppressive monocytes in experimental autoimmune encephalomyelitis. *J. Immunol.* **179**, 5228–5237.
28. Haile, L. A., von Waselewski, R., Gamrekeshvili, J., Kruger, C., Bachmann, O., Westendorf, A. M., Buer, J., Liblau, R., Manns, M. P., Korangy, F., Greten, T. F. (2008) Myeloid-derived suppressor cells in inflammatory bowel disease: a new immunoregulatory pathway. *Gastroenterology* **135**, 871–881, 881.e1–881.e5.
29. Fujii, W., Ashihara, E., Hirai, H., Nagahara, H., Kajitani, N., Fujioka, K., Murakami, K., Seno, T., Yamamoto, A., Ishino, H., Kohno, M., Maekawa, T., Kawahito, Y. (2013) Myeloid-derived suppressor cells play crucial roles in the regulation of mouse collagen-induced arthritis. *J. Immunol.* **191**, 1073–1081.
30. Yin, B., Ma, G., Yen, C. Y., Zhou, Z., Wang, G. X., Divino, C. M., Casares, S., Chen, S. H., Yang, W. C., Pan, P. Y. (2010) Myeloid-derived suppressor cells prevent type 1 diabetes in murine models. *J. Immunol.* **185**, 5828–5834.
31. Kerr, E. C., Raveney, B. J., Copland, D. A., Dick, A. D., Nicholson, L. B. (2008) Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations. *J. Autoimmun.* **31**, 354–361.
32. Lee, P. Y., Weinstein, J. S., Nacionales, D. C., Scumpia, P. O., Li, Y., Butfiloski, E., van Rooijen, N., Moldawer, L., Satoh, M., Reeves, W. H. (2008) A novel type I IFN-producing cell subset in murine lupus. *J. Immunol.* **180**, 5101–5108.
33. Xu, Y., Lee, P. Y., Li, Y., Liu, C., Zhuang, H., Han, S., Nacionales, D. C., Weinstein, J., Mathews, C. E., Moldawer, L. L., Li, S. W., Satoh, M., Yang, L. J., Reeves, W. H. (2012) Pleiotropic IFN-dependent and -independent effects of IRF5 on the pathogenesis of experimental lupus. *J. Immunol.* **188**, 4113–4121.
34. Nacionales, D. C., Kelly-Scumpia, K. M., Lee, P. Y., Weinstein, J. S., Lyons, R., Sobel, E., Satoh, M., Reeves, W. H. (2007) Deficiency of the type I interferon receptor protects mice from experimental lupus. *Arthritis Rheum.* **56**, 3770–3783.
35. Grainger, J. R., Wohlfert, E. A., Fuss, I. J., Bouladoux, N., Askenase, M. H., Legrand, F., Koo, L. Y., Brenchley, J. M., Fraser, I. D., Belkaid, Y. (2013) Inflammatory monocytes regulate pathologic responses to commensals during acute gastrointestinal infection. *Nat. Med.* **19**, 713–721.
36. Iwata, Y., Furuichi, K., Kitagawa, K., Hara, A., Okumura, T., Kokubo, S., Shimizu, K., Sakai, N., Sagara, A., Kurokawa, Y., Ueha, S., Matsushima, K., Kaneko, S., Wada, T. (2010) Involvement of CD11b+ GR-1 low cells in autoimmune disorder in MRL-Fas lpr mouse. *Clin. Exp. Nephrol.* **14**, 411–417.
37. Jutila, M. A., Kroese, F. G., Jutila, K. L., Stall, A. M., Fiering, S., Herzenberg, L. A., Berg, E. L., Butcher, E. C. (1988) Ly-6C is a

- monocyte/macrophage and endothelial cell differentiation antigen regulated by interferon-gamma. *Eur. J. Immunol.* **18**, 1819–1826.
38. Sunderkötter, C., Nikolic, T., Dillon, M. J., Van Rooijen, N., Stehling, M., Drevets, D. A., Leenen, P. J. (2004) Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J. Immunol.* **172**, 4410–4417.
39. Ioannou, M., Alissafi, T., Lazaridis, I., Deraos, G., Matsoukas, J., Gravanis, A., Mastorodemos, V., Plaitakis, A., Sharpe, A., Boumpas, D., Verginis, P. (2012) Crucial role of granulocytic myeloid-derived suppressor cells in the regulation of central nervous system autoimmune disease. *J. Immunol.* **188**, 1136–1146.
40. Cripps, J. G., Gorham, J. D. (2011) MDSC in autoimmunity. *Int. Immunopharmacol.* **11**, 789–793.
41. Bronte, V., Serafini, P., De Santo, C., Marigo, I., Tosello, V., Mazzoni, A., Segal, D. M., Staib, C., Lowel, M., Sutter, G., Colombo, M. P., Zanovello, P. (2003) IL-4-induced arginase 1 suppresses alloreactive T cells in tumor-bearing mice. *J. Immunol.* **170**, 270–278.
42. Kusmartsev, S., Gabrilovich, D. I. (2005) STAT1 signaling regulates tumor-associated macrophage-mediated T cell deletion. *J. Immunol.* **174**, 4880–4891.
43. Campbell, E. L., Bruyninckx, W. J., Kelly, C. J., Glover, L. E., McNamee, E. N., Bowers, B. E., Bayless, A. J., Scully, M., Saeedi, B. J., Golden-Mason, L., Ehrentraut, S. F., Curtis, V. F., Burgess, A., Garvey, J. F., Sorensen, A., Nemenoff, R., Jedlicka, P., Taylor, C. T., Kominsky, D. J., Colgan, S. P. (2014) Transmigrating neutrophils shape the mucosal microenvironment through localized oxygen depletion to influence resolution of inflammation. *Immunity* **40**, 66–77.
44. Josefowicz, S. Z., Lu, L. F., Rudensky, A. Y. (2012) Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* **30**, 531–564.
45. Huang, B., Pan, P. Y., Li, Q., Sato, A. I., Levy, D. E., Bromberg, J., Divino, C. M., Chen, S. H. (2006) Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res.* **66**, 1123–1131.
46. Hoechst, B., Ormandy, L. A., Ballmaier, M., Lehner, F., Krüger, C., Manns, M. P., Greten, T. F., Korangy, F. (2008) A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology* **135**, 234–243.
47. Mildner, A., Mack, M., Schmidt, H., Brück, W., Djukic, M., Zabel, M. D., Hille, A., Priller, J., Prinz, M. (2009) CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system. *Brain* **132**, 2487–2500.
48. King, I. L., Dickendesher, T. L., Segal, B. M. (2009) Circulating Ly-6C+ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. *Blood* **113**, 3190–3197.
49. Westendorf, A. M., Fleissner, D., Deppenmeier, S., Gruber, A. D., Bruder, D., Hansen, W., Liblau, R., Buer, J. (2006) Autoimmune-mediated intestinal inflammation-impact and regulation of antigen-specific CD8+ T cells. *Gastroenterology* **131**, 510–524.
50. Thaxton, J. E., Liu, B., Zheng, P., Liu, Y., Li, Z. (2014) Deletion of CD24 impairs development of heat shock protein gp96-driven autoimmune disease through expansion of myeloid-derived suppressor cells. *J. Immunol.* **192**, 5679–5686.

KEY WORDS:

myeloid-derived suppressor cell · regulatory T cell · prostaglandin E2 · nitric oxide synthase · IL-10