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Dexamethasone inhibits in vivo tumor growth by the alteration of bone marrow CD11b$^+$ myeloid cells

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ABSTRACT

Inflammation is closely associated with tumor growth, which is mediated by the activation of bone marrow-derived CD11b$^+$ cells. Here, we investigated whether anti-inflammatory dexamethasone (Dex), a synthetic glucocorticoid (GC), could regulate tumor growth and CD11b$^+$ myeloid bone marrow cells (BMCs) in lymphocyte (R1), monocyte (R2) and granulocyte (R3) regions of FSC-SSC dot plot. The growth of B16F10 mouse melanoma tumor was inhibited in Dex-injected group. Lung metastasis was decreased and the lifespan was elongated in Dex-injected mice with tumor resection. Intravenous injection of B16F10 cells increased the percentage of CD11b$^+$ myeloid BMCs in R1 and R2 regions from 3 h to 72 h. In contrast, little changes in the percentage of CD11b$^+$ myeloid BMCs were detected in R3 region. Among CD11b$^+$ myeloid BMCs, the percentage of CD11b$^+$Gr-1$^+$ cells was increased in R1, R2 and R3 regions. Absolute number of CD11b$^+$ and CD11b$^+$Gr-1$^+$ cells was enhanced in R1 region from 3 h to 72 h. B16F10 tumor growth was significantly increased by intravenous injection of CD11b$^+$ BMCs. Tumor-bearing mice showed an increase in the percentage of CD11b$^+$ myeloid BMCs in R2 region and CD11b$^+$Gr-1$^+$ cells in R2 and R3 regions, which are reduced by intravenous injection with Dex. Absolute number of CD11b$^+$Gr-1$^+$ cells was enhanced in R2 and R3 regions. Tumor growth was significantly inhibited by intravenous injection of BMCs collected from Dex-treated tumor-bearing mice. Taken together, data demonstrate that tumor regression by Dex was resulted from the alteration of CD11b$^+$ myeloid BMCs and their inhibitory function to tumor growth. It suggests that CD11b$^+$ myeloid BMCs could regulate antitumor efficacy of GCs such as Dex.

1. Introduction

Tumor growth is associated with the activation of bone marrow cells (BMCs) [1]. In addition, bone marrow-derived cells (BMDCs) enhance tumor angiogenesis and growth. The role of BMDCs is not only limited to the growth of primary tumors but also metastasis [2,3]. BMDCs are the targets as well as the tool for cancer therapy [4]. Impairment of tumor growth could be restored by transplantation of BMCs in which CD11b$^+$ myeloid BMCs are a major population [5]. Tumor-infiltrated CD11b$^+$ myeloid cells also promote angiogenesis [6,7] by expressing various proangiogenic and chemoattractant molecules [8].

CD11b (Mac-1, αMβ2) is expressed on monocytes/macrophages and granulocytes [9]. CD11b has been shown to mediate many functions of myeloid cells, including adhesion, migration, chemotaxis, phagocytosis, and respiratory burst activity [9]. CD11b-neutralizing monoclonal antibodies inhibit the recruitment of myeloid cells into tumor microenvironment [10]. Therefore, CD11b$^+$ myeloid BMCs could be useful target as a cancer therapy.

Among CD11b$^+$ myeloid BMCs, CD11b$^+$Gr-1$^+$ myeloid-derived suppressor cells (MDSC) are detected in sizeable numbers but small number of these cells (<4 %) are found in secondary lymphoid organs of healthy mice [11]. MDSC can be accumulated in the blood and lymphoid organs of tumor-bearing mice [12]. MDSC have been clearly linked to dysfunctional immune responses such as the alteration of CD8$^+$ T-cell responsiveness to both antigen-specific and antigen-independent stimulation [13]. MDSC represent hematopoietic responses with a negative immune regulatory activity to cancer [14].

Glucocorticoids (GCs) have been broadly used to induce apoptotic cell death in lymphoma [15] and to treat chronic lymphocytic leukemia [16]. In contrast, GC signaling might also contribute to cancer progression [15] such as breast cancer cells [17] and cervical cancer cells [18]. GCs stimulate anti-apoptotic gene expression and antagonize the ability of cancer cytotoxics to effectively induce cell death [19]. However, little has been known about GC-mediated effect on the relationship between tumor growth and CD11b$^+$ myeloid BMCs.

In our study, we examined whether GCs could affect tumor growth through the changes in CD11b$^+$ myeloid BMC population and the function of BMCs using dexamethasone (Dex), a synthetic GC. Our data demonstrate that tumor growth was increased by CD11b$^+$ myeloid BMCs and it was inhibited by BMCs collected from Dex-treated

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tumor-bearing mice. It suggests that $CD11b^+$ myeloid BMCs could be a regulator to control antitumor effect on various types of tumor cells.

2. Materials and methods

2.1. Mice and reagents

Six weeks old female C57BL/6J mice were purchased from Daehan Biolink (Chungju, Korea). The companion of five mice was housed in the transparent acrylic cage. Mice were maintained in the pathogen-free authorized facility in Sejong University where the temperature was maintained at 20–22 °C and the humidity at 50–60%; there was a 12 h dark/light cycle. All experiments using mice were carried out in strict accordance with the guidelines of the recommendations in the Guide for the Care and Use of Laboratory Animals of 'Animal and Plant Quarantine Agency', Korea. The protocol was approved by the Institutional Animal Care and Use Committee, Sejong University (Permit Number: SJ-20120604). All surgery was performed under the anesthesia with a mixture of Zoletil™ (Tiletamine + Zolazepam) and Rompun (Xylaine), and all efforts were made to minimize suffering.

Dexamethasone was purchased from Sigma chemical company (St. Louis, MO). FITC-conjugated anti-CD11b and PE-conjugated anti-Gr-1 antibodies were obtained from BD Biosciences (San Jose, CA). MACS™ cell isolation kit using anti-CD11b antibodies was purchased from Miltenyi Biotec (San Diego, CA). Except where indicated, all other materials are obtained from the Sigma chemical company (St. Louis, MO).

2.2. Cell cultures

B16F10 mouse melanoma cells were obtained from the Korea Institute of Radiological and Medical Science (KIRMS) cell bank (Seoul, Korea). B16F10 cells were maintained and cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, NY), supplemented with 5% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY), 2 mM L-glutamine, 100 units/ml penicillin and 100 units/ml streptomycin (GIBCO, Grand Island, NY). Cells were incubated in an incubator at 37 °C in an atmosphere of 5 % CO_2 in air.

2.3. In vivo tumor growth

Mice were subcutaneously implanted with 2×10^5 B16F10 mouse melanoma cells. Tumor growth was monitored by the measurement of short axis and long axis of tumor mass with vernier calipers (Mizuyo, Japan). Tumor volume was calculated by the multiplication of (long axis)/2 and (short axis)^2.

2.4. In vivo spontaneous tumor metastasis [20] and survival study

For the experimental spontaneous lung metastasis formation, B16F10 cells were cultivated in vitro in log phase. C57BL/6 mice were injected with 2×10^5 B16F10 cells suspended in 0.2 ml sterile PBS, subcutaneously. When tumor volume became 750–1000 mm^3, tumors were resected from tumor-bearing mice and the wound was sutured under the anesthetic condition. Then, the mice were maintained for 10–12 weeks. To observe lung metastasis, the mice were sacrificed by cervical dislocation and lung metastasis number was assessed by counting tumor colony under a dissection microscope.

We also measured lifespan of animals after tumor resection as follows. Briefly, the condition of mice was monitored whether they stopped breathing without lung inflation every 12 h during the maintenance. When the mice were died as a direct result of the intervention, the lifespan of each mouse was counted from the day of tumor resection. This experimental procedure was kept until two mice were left in any group out of between control and experimental groups. Mice left with good or bad breathing were humanely euthanized by cervical dislocation. Survival probability was represented by Kaplan Meier curve.

2.5. Flow cytometric analyses

Aliquots of 1.0×10^6 cells from each tissue from both control or cancer cell-injected mice were suspended in 2 % FCS containing RPMI 1640. Cells were then treated with FITC-conjugated anti-CD11b and PE-conjugated anti-Gr-1 antibodies. Each cell suspension was incubated with antibodies for 20 min on ice. Thereafter, the cells were washed with medium and analyzed by CELLQuest™ software in FACScalibur™ (Becton Dickinson, Franklin Lakes, NJ). A minimum of 10,000 events were analyzed/cell population of interest.

2.6. Statistical analyses

Experimental differences were tested for statistical significance using ANOVA and Students' t-test. P value of <0.05 or 0.01 was considered to be significant.

3. Results

3.1. Dexamethasone decreased in vivo tumor growth and lung metastasis

Given that inflammation is closely associated with tumor growth, angiogenesis and metastasis [21–23], we examined whether dexamethasone (Dex), a synthetic glucocorticoid (GC), could regulate tumor growth. When tumor-bearing mice were intravenously injected with Dex, tumor growth was inhibited significantly (Fig. 1A). Tumor mass and weight were consistent to the changes in tumor volume (Fig. 1B). Data demonstrate that Dex could affect tumor growth in tumor-bearing mice. When Dex was injected into mice with tumor resection, we observed that lung metastasis was lower in Dex-treated mice as compared to Dex-untreated control group (Fig. 1C and D). In addition, the lifespan was elongated by the injection with Dex into mice with tumor resection (Fig. 1E and F). It suggests that Dex could be effective therapeutics for the inhibition of solid tumor growth and metastasis.

3.2. Tumor growth was enhanced by bone marrow $CD11b^+$ cells

Given that tumor growth is mediated by the activation of bone marrow-derived $CD11b^+$ cells [2,3,5], we examined the changes in $CD11b^+$ cells by tumor cells in various immunological tissues. When cells in immunological tissues were analyzed by flow cytometer, $CD11b^+$ myeloid cell population was the highest in BM than other immunological tissues (Fig. 2A). When B16F10 cells were intravenously injected into mice, we observed three populations in FSC-SSC dot plot gated into R1, R2, and R3 regions (Fig. 2B). The cellular subpopulations in R1, R2 and R3 are lymphocytes, monocytes, and granulocytes, respectively [24]. No changes in the percentage of FSC-SSC population were detected in three regions as compared in between before and after intravenous injection of tumor cells (Supplementary Fig. 1). When BMCs were analyzed in each region, an increase in $CD11b^+$ or $CD11b^+Gr\text{-}1^+$ cells was detected in R1 and R2 regions at 12 h after the intravenous injection of B16F10 cells (Fig. 2C). In addition, while the percentage of $CD11b^+$ myeloid BMCs was also increased, time dependently in R1 and R2 regions, little changes were detected in R3 region (Fig. 2D). Among $CD11b^+$ myeloid BMCs, the percentage of $CD11b^+Gr\text{-}1^+$ cells was increased in R1, R2 and R3 regions of B16F10-injected mouse BMCs (Fig. 2E). We also examined the absolute number of each cell population in BMCs from 3 h to 72 h after intravenous injection of B16F10 cells into mice (Table 1). The absolute number of BMCs in femur and tibia was decreased by B16F10 tumor cells. The absolute cell number in FSC-SSC dot plot was reduced in R1, R2, and R3 regions of tumor-bearing mouse BMCs as compared to control. While the absolute