Local and systemic inflammation triggers different outcomes of tumor growth related to infiltration of anti-tumor or pro-tumor macrophages

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Abstract

Background: Previous evidence suggests inflammation may be a double-edged sword with cancer-promoting and cancer suppressing function. In this study, we explore the impact of local and systemic inflammation on cancer growth.

Methods: Female BALB/C mice were subcutaneously implanted with foreign body (plastic plates) to build up a local inflammation and intraperitoneally injected with PolyIC or lipopolysaccharides (LPS) to build up a systemic inflammation, followed by subcutaneous injection of 5×10^5 colon cancer cells. Immunohistochemistry and enzyme linked immunosorbent assay were utilized to detect the Ki67 and interleukin (IL) 6, IL-1β, and monocyte chemoattractant protein-1 expression in the tumor tissues and serum, respectively. The distributions of immune cells and expression of toll-like receptors (TLRs) were evaluated by flow cytometry (FCM) and quantitative real time-polymerase chain reaction.

Results: The results showed that local inflammation induced by foreign body implantation suppressed tumor growth with decreased tumor weight ($P = 0.001$), volume ($P = 0.004$) and Ki67 index ($P < 0.001$). Compared with the control group, myeloid-derived suppressive cells sharply decreased ($P = 0.040$), while CD4$^+$ T cells slightly increased in the tumor tissues of the group of foreign body-induced local inflammation ($P = 0.035$). Moreover, the number of M1 macrophages ($P = 0.040$) and expression of TLRs, especially TLR3 ($P < 0.001$) and TLR4 ($P < 0.001$), were significantly up-regulated in the foreign body group. Contrarily, tumor growth was significantly promoted in LPS or PolyIC-induced systemic inflammation ($P = 0.009$ and 0.006). FCM results showed M1 type macrophages ($P = 0.017$ and 0.006) and CD8$^+$ T cells ($P = 0.031$ and 0.023) were decreased, while M2 type macrophages ($P = 0.002$ and 0.007) were significantly increased in tumor microenvironment of LPS or PolyIC-induced systemic inflammation group. In addition, the decreased expression of TLRs was detected in LPS or PolyIC group.

Conclusions: The foreign body-induced local inflammation inhibited tumor growth, while LPS or PolyIC- induced systemic inflammation promoted tumor growth. The results suggested that the different outcomes of tumor growth might be attributed to the infiltration of anti-tumor or pro-tumor immune cells, especially M1 or M2 type macrophages into tumor microenvironment.

Keywords: Inflammation; Cancer; Macrophage; Toll-like receptor

Introduction

Inflammation is an adaptive response induced by endogenous or exogenous stimulation and is coordinated by multiple cells, inflammatory molecules, receptors and others.[1] Exogenous stimuli consist of microbial factors (bacteria, virus and nematodes) and non-microbial factors (foreign bodies, radiation and mineral fibers), while endogenous stimulators are usually derived from stressed, damaged or malfunctioned tissues and cells, and degradation products of extracellular matrix.[2,3] Stimulators of inflammation trigger immune or tissue cells to secret numerous inflammatory mediators (cytokines, proteolytic enzymes, chemokines, and free radicals), which in turn affect the tissues and cells and inflammatory response[4,5]

Some epidemiological studies showed that inflammation increased the incidence of various types of cancer, such as bladder cancer, cervical cancer, gastric cancer, and bowel cancer.[6] According to statistics, chronic infection and inflammation caused about 25% of cancer cases worldwide, and patients with inflammation had a significant increased risk of cancer. Apparently, inflammation not only directly initiates cancer, but also indirectly contrib-

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utes to the progression and metastasis of cancer.[7,8] In some situation, local inflammation may serve as a "fertile soil" to allow cancer growth and metastasis[9,10] For instance, physical trauma, which induced local inflammation and wound healing reaction, created an inflammatory milieu of cytokines, chemokines, and growth factors that augmented angiogenesis and favored cancer invasion and evasion of immune surveillance.[11,12] Carrageenan- induced acute local inflammation also promoted cancer growth by generating the increased prostanoids and pro-inflammatory cytokines.[13] Nevertheless, inflammation caused by toll-like receptor (TLR) agonists or *Mycobacterium bovis,* can be clinically used to suppress the growth and metastasis of cancer.[14,15] In patients with colon cancer, local inflammation predicts a better progno- sis,[16,17] while systemic inflammation is opposite[18,19] All evidences suggest that inflammation may be a doubleedged sword with cancer-promoting and cancer-suppressing function. Recently, more and more studies have found that the effects of inflammation on cancer are quite different according to the scope of inflammation. However, it is uncertain how local and systemic inflammation triggers different outcomes of tumor growth.

Thus, in the present study, we used foreign body (plastic plates) to build up a local inflammation and intraperitoneal injection of TLR3 agonist (PolyIC) or TLR4 agonist (LPS) to build up a systemic inflammation, and assessed the impact of both local and systemic inflammation on cancer.

Methods

Ethical approval

All of the animal protocols were approved by the Institutional Animal Care and Use Committee of Nanjing First Hospital, Nanjing Medical University (No. DW20200208) and conformed to the guidelines published by the National Institutes of Health.

Cell culture

CT26 colon cancer line was purchased from KeyGen BioTech (Nanjing, China) and cultured in Roswell Park Memorial Institute (RPMI)-1640 containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and 1% volume/volume (v/v) penicillin and streptomycin (Invitrogen) at 37°C in a 5% CO_2 incubator.

Tumor mouse model under local or systemic inflammation

Female BALB/C mice (5–6 weeks) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). The mouse tumor model under local inflammation was built as follows: plastic plates of foreign body were subcutaneously implanted into the right flank of female BALB/C mice (5–6 weeks).[20] Five days later, 5×10^5 CT26 cells were subcutaneously injected at the site of plastic plates. The mouse tumor model under systemic inflammation was built by intraperitoneal injection of LPS (Sigma-Aldrich, St. Louis, MO, USA; 2 mg/kg, every other day for a week) or PolyIC (InvivoGen, San Diego, CA, USA; 2.5 mg/kg, every other day for two weeks) to female

BALB/C mice, followed by subcutaneous injection of 5×10^5 CT26 cells on the right flank. Indexes like body weight and tumor size were recorded every other day. Tumor volume was calculated by the formula: volume (V) = length (L) × width (W)^2 × 1/2.

Analysis of tumor-infiltrating immunocytes

Tumor tissues were peeled off from the mice, cut into pieces and digested with RPMI1640 medium containing 5% FBS, 2 mg/mL collagenase IV (Sigma-Aldrich, St. Louis, MO, USA), and 5 U/mL DNase I (Sigma-Aldrich) for 20 min by a 37°C shaker. After being neutralized with RPMI 1640 medium containing 5% FBS, cell suspension was filtered through a 70-μm nylon mesh into a centrifuge tube to get single cell suspensions. Scissors and 1 mL sterile syringes were used to separate the femurs and tibias and obtain the bone marrow cells. Then, the cells were lysed with red blood cell lysate, filtered through gauze, and resuspended in phosphate-buffered saline. For immunocyte detection, the following anti-mouse antibodies were used: CD45-phycoerythrin (PE) (103106, BioLegend, SanDiego, CA, USA), CD45-allophycocyanin (APC) (17-0451-83, eBioscience, CA, USA), CD11b-APC (101212, BioLegend), CD11b-peridinin-chlorophyll-protein complex (45-011282, eBioscience), glutathione reductase-1-fluorescein isothiocyanate (108406, BioLegend), F4/80-fluorescein isothiocyanate (FITC) (11-480182, eBioscience), inducible nitric oxide synthase-PE (125920-82, eBioscience), CD206-PE (12-2061-82, eBioscience), CD206-APC (17-2061-82, eBioscience), CD3-APC (17-0031-81, eBioscience), CD8-FITC (11-0081-81, eBioscience), CD4-Alexa Fluor 488 (100423, BioLegend). Stained cells were detected by a fluorescence-activated cell sorting Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and data were analyzed using FlowJo software (TreeStar, Ashland, OR, USA).

Isolation of total RNA and quantitative real time-polymerase chain reaction (qRT-PCR)

Total RNA from cells isolated from the tumor tissues was extracted by TRIzol regent (Invitrogen) and reverse-transcribed to complementary DNA (cDNA) by HiScript II Q RT SynthesisMix Kit (Vazyme, Nanjing, China) following the manufacturer's protocol. Then, SYBR Green SuperMix reagent (Bio-Rad, Hercules, CA, USA) was applied to polymerase chain reaction (PCR) quantification of cDNA. The expression of targeted genes was quantitatively calculated based on the ratio of target genes to the internal reference gene (glyceraldehyde-3-phosphate dehydrogenase) using the $2^{-\Delta\Delta Ct}$ method. Primers used in this study were displayed in Table 1.

Enzyme linked immunosorbent assay (ELISA)

First, blood was obtained from the eye socket of mice and allowed to stand at room temperature for 30 min. The blood serum was collected by centrifugation at 400 g for 20 min. The expression of interleukin (IL)-6, IL-1β, and monocyte chemoattractant protein-1 (MCP-1) was determined according to the instructions of the mouse ELISA kit (Neobioscience, Shanghai, China).

Table 1: Primer sequences used in qRT-PCR.

Gene name	Primers	Sequences (5′ to 3′)
GAPDH	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	TGTAGACCATGTAGTTGAGGTCA
TLR2	Forward	CACCACTGCCCGTAGATGAAG
	Reverse	AGGGTACAGTCGTCGAACTCT
TLR3	Forward	AAAATCCTTGCGTTGCGAAGT
	Reverse	TGTTCAAGAGGAGGGCGAATAA
TLR4	Forward	AAATGCACTGAGCTTTAGTGGT
	Reverse	TGGCACTCATAATGATGGCAC
TLR5	Forward	TGGGGACCCAGTATGCTAACT
	Reverse	CCACAGGAAAACAGCCGAAGT
TLR6	Forward	TGAGCCAAGACAGAAAACCCA
	Reverse	GGGACATGAGTAAGGTTCCTGTT
TLR7	Forward	ATGTGGACACGGAAGAGACAA
	Reverse	ACCATCGAAACCCAAAGACTC
TLR8	Forward	GCCAAACAACAGCACCCAAAT
	Reverse	AGGCAACCCAGCAGGTATAGT
TLR9	Forward	ATGGTTCTCCGTCGAAGGACT
	Reverse	GAGGCTTCAGCTCACAGGG

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR: Quantitative real time-polymerase chain reaction; TLR: Toll-like receptor.

Immunohistochemistry

Four percent paraformaldehyde (Servicebio, Wuhan, China) and paraffin were used to fix and embed the tumor tissues from different groups of mice. Ki67 antibody was selected as the primary antibody. All sections were imaged and analyzed by the light microscope and Image J software (National Institutes of Health, USA), respectively.

Hematoxylin-eosin (H&E) staining

The paraffin sections were deparaffinized to water, stained with hematoxylin (Servicebio) and eosin (Servicebio), and dehydrated by ethanol (Servicebio). Finally, the slices were sealed with neutral gum and observed under the microscope.

Statistical analysis

Prism 6 (GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analysis. Student's t-test or one-way anaylsis of variance (ANOVA) followed by Dunnett multiple comparison test or Tukey multiple comparision test was used to analyze differences among groups. Differences were considered significant as $P < 0.05$ and highly significant as $P < 0.01$.

Results

Tumor growth is suppressed under condition of foreign body-induced local inflammation

To investigate the effect of local inflammation on colon cancer growth, we subcutaneously implanted a plastic plate as foreign body on the right flank of female BALB/C mice, and then inoculated with CT26 cells in the same site [Figure 1A]. As seen in Figures 1B–1D, foreign body-induced local inflammation slightly suppressed tumor growth as evidenced by decreased average tumor weight ($P = 0.001$) and volume ($P = 0.004$). Immunohistochemical staining of Ki67 showed foreign body reduced tumor proliferation to a great extent [Figures 1E and 1F]. Of note, foreign body implantation indeed induced the elevated level of IL6 in the mouse serum ($P = 0.002$) [Figure 1G], indicating inflammation occurrence. However, the expression of IL-1β ($P = 0.526$) and MCP-1 ($P = 0.872$) did not change significantly in the serum of mice with local inflammation [Figure 1G]. H&E images of tumor tissues showed the number of tumor cells in the mice with local inflammation was less than that in the control group [Figure 1H].

Local inflammation just affects the development of immature myeloid-derived cells

To assess the alteration of bone marrow, some immune cells from the bone marrow cells were obtained and analyzed. As shown in Figure 2, the proportion of myeloid-derived suppressor cells (MDSCs; CD11b⁺Gr-1⁺; $P = 0.010$) in the bone marrow cells was lower in the group of foreign body [Figure 2A and 2B], although the percentages of granulocyte-MDSCs (G-MDSCs, $P = 0.051$) and monocytic-MDSCs (M-MDSCs, $P = 0.771$) stayed the same [Figure 2C and 2D]. In addition, the total macrophages [Figure 2E and 2F, CD11b⁺F4/80⁺, $P = 0.196$], M1 (CD11b⁺F4/80⁺iNOS⁺, $P = 0.285$) and M2 macrophages (CD11b⁺F4/80⁺CD206⁺, $P = 0.610$) had not changed significantly [Figure 2G]. The number of CD4⁺ T cells ($P = 0.238$) was similar in the two groups, while the number of CD8⁺ T cells ($P = 0.004$) was higher in the local inflammation group than control group [Figure 2H]. These results suggested that foreign body-induced local inflammation might mainly affect the development of immature myeloid-derived cells.

The supression of tumor growth is related to infiltration of M1 type macrophages into tumor microenvironments under local inflammation

To assess the alteration of tumor microenvironment, some immune cells from tumor tissues were obtained and analyzed. The proportions of MDSCs (CD45⁺CD11b⁺Gr-1⁺, $P = 0.040$) in tumor tissues were significantly decreased in the foreign body group, accompanied with decreased percentages of G-MDSCs ($P = 0.009$) and M- MDSCs ($P = 0.001$) [Figures 3A–3D]. Total macrophages (CD45⁺CD11b⁺F4/80⁺, $P = 0.013$), especially M1-type macrophages (CD45⁺CD11b⁺F4/80⁺iNOS⁺, $P = 0.004$) were significantly increased in foreign body-induced group, while the numbers of M2 type macrophages (CD45⁺CD11b⁺F4/80⁺CD206⁺, $P = 0.731$) were similar between the two groups [Figures 3E and 3F]. In addition, CD4⁺ T cells (CD45⁺CD3⁺CD4⁺, $P = 0.035$) slightly increased in the group with foreign body, while CD8⁺ T cells (CD45⁺CD3⁺CD8⁺, $P = 0.760$) did not have an statistical difference between the two group [Figure 3G]. To explore the potential mechanism, single cells were extracted from tumor tissues and analyzed by qRT-PCR. We surprisingly found that the expression of TLRs,
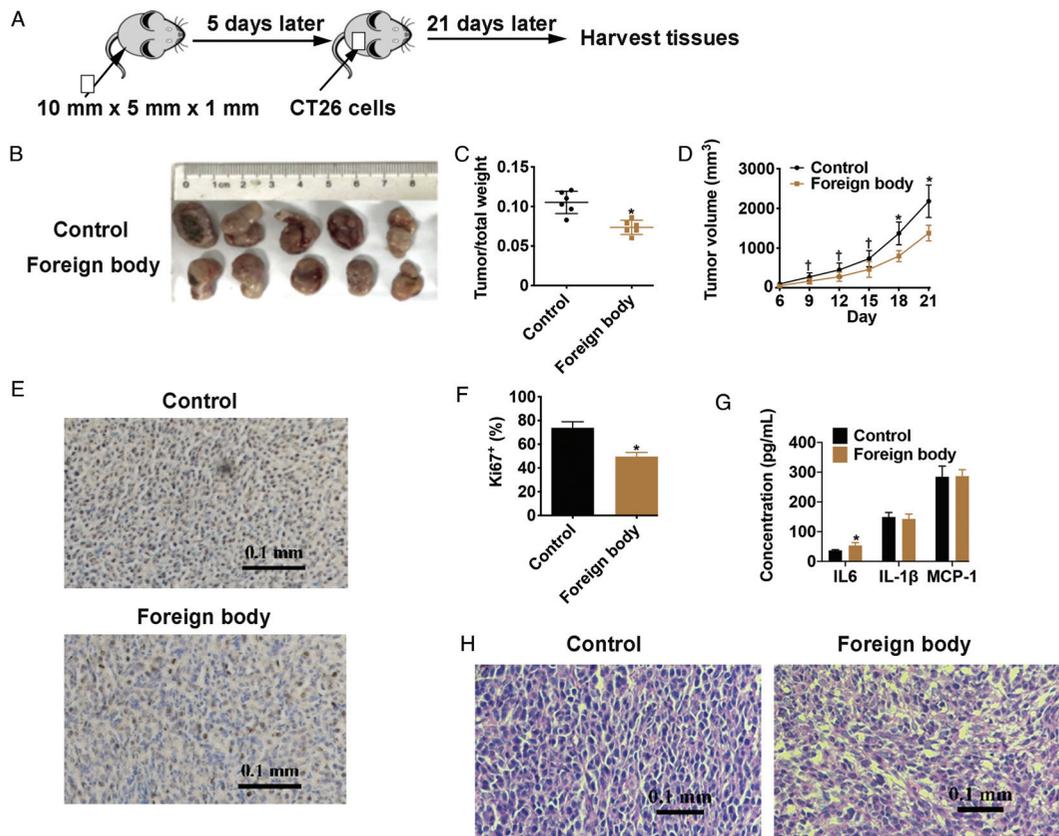
Figure 1: Local inflammation induced by subcutaneous implantation of plastic plates inhibits tumor growth. (A) The scheme of mouse model with local inflammation. (B–D) Photograph of dissected tumor tissues (B) and quantitative data of tumor weight (C) and volume (D) from mice with the indicated treatment. (E–F) Immunohistochemistry photograph (E) and quantitative data (F) of Ki67 staining of tumor tissues from mice with the indicated treatment. (G) The expression of IL6, IL-1 β, and MCP-1 in the blood serum from mice with the indicated treatment. (H) H&E images of tumor tissues from mice with the indicated treatment. $^*P < 0.01$, $^†P < 0.05$ *vs.* control. H&E: Hematoxylin and eosin; IL: Interleukin; MCP-1: Monocyte chemoattractant protein-1.
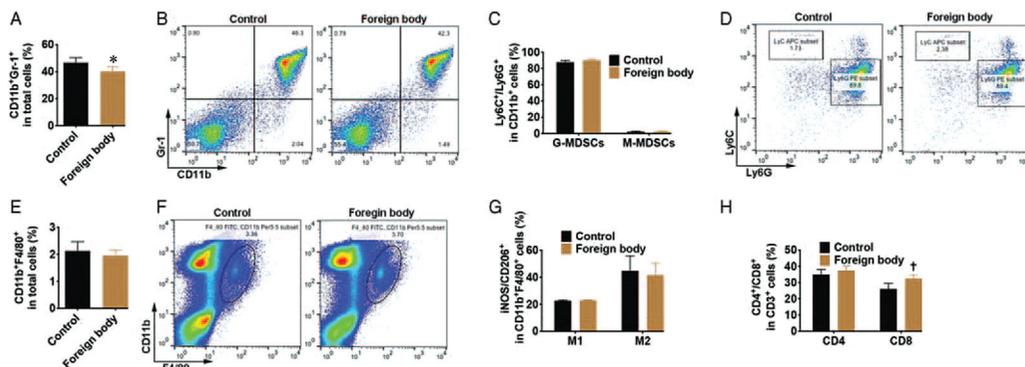

Figure 2: Local inflammation affects the development of immature myeloid-derived cells in the bone marrow cells *in vivo.* (A–B) Quantitative data of the proportions (A) and FCM photographs (B) of MDSCs in the bone marrow cells from mice with indicated treatments. (C–D) Quantitative data of the proportions (C) and FCM photographs (D) of subgroups of MDSCs in the bone marrow cells from mice with indicated treatments. (E–F) Quantitative data of the proportions (E) and FCM photographs (F) of macrophages in the bone marrow cells from mice with indicated treatments. (G–H) Quantitative data of the proportions of subgroups of macrophages (G) and CD4$^+$ and CD8$^+$ T cells (F) in the bone marrow cells from mice with indicated treatments. $^*P < 0.01$, $^†P < 0.05$ *vs.* control. FCM: Flow cytometry; G-MDSCs: Granulocyte-myeloid-derived suppressor cells; Gr-1: Glutathione reductase-1; iNOS: Inducible nitric oxide synthase; MDSCs: Myeloid-derived suppressor cells; M-MDSCs: Monocytic-myeloid-derived suppressor cells.

especially TLR3 ($P < 0.001$) and TLR4 ($P < 0.001$), was significantly elevated in the foreign body group [Figure 3H]. These data indicated that the supression of tumor growth might be related to infiltration of antitumor M1 type macrophages into tumor microenvironments under foreign body-induced local inflammation.

Tumor growth is promoted under condition of LPS or PolyIC-induced systemic inflammation

Next, we wanted to explore the effect of systemic inflammation on tumor growth. PolyIC and LPS were intraperitoneally injected to induce systemic inflammation and then CT26 cells were subcutaneously injected at the
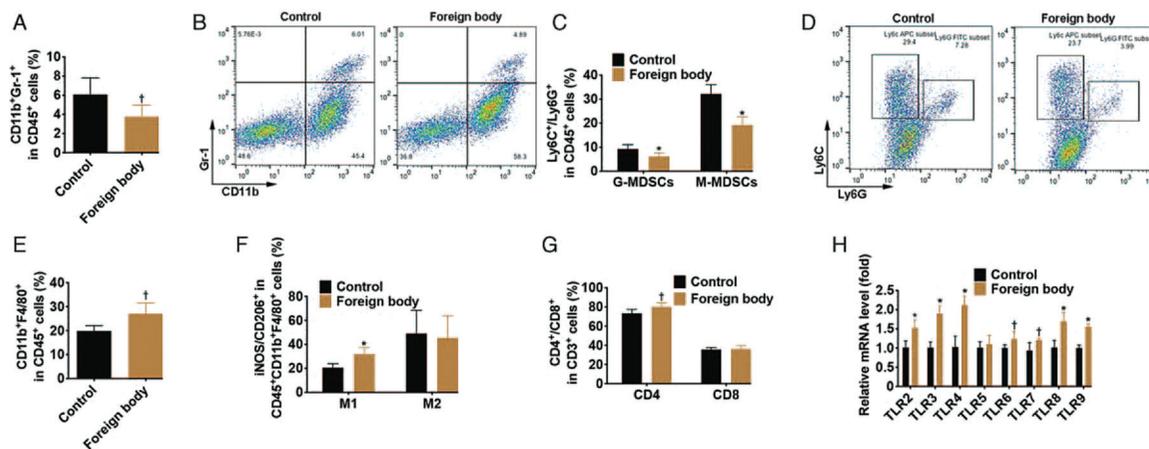
Figure 3: M1 type macrophages infiltrates into tumor microenvironments under local inflammation *in vivo*. (A–B) Quantitative data of the proportions (A) and FCM photographs of MDSCs (B) in the tumor tissues from mice with indicated treatments. (C–D) Quantitative data of the proportions (C) and FCM photographs (D) of subgroups of MDSCs in the tumor tissues from mice with indicated treatments. (E–G) Quantitative data of the proportions of macrophages (E), subgroups of macrophages (F), and CD4+ and CD8+ T cells (G) in the tumor tissues from mice with indicated treatments. (H) Single cells were extracted from the tumor tissues and used for qRT-PCR to determine the expression of targeted genes. *P < 0.01, †P < 0.05 *vs.* control. FCM: Flow cytometry; G-MDSCs: Granulocyte-myeloid-derived suppressor cells; Gr-1: Glutathione reductase-1; iNOS: Inducible nitric oxide synthase; MDSCs: Myeloid-derived suppressor cells; M-MDSCs: Monocytic-myeloid-derived suppressor cells; qRT-PCR: Quantitative real time-polymerase chain reaction; TLR: Toll-like receptor.

right flank of female BALB/C mice [Figure 4A]. The results showed that the systemic inflammation sharply promoted tumor growth, as evidenced by the increased average tumor weight ($P = 0.004$ and 0.002) and volume ($P = 0.009$ and 0.006) [Figure 4B–4D]. The systemic inflammation also enhanced the proliferation of tumor cells detected by Ki67 staining (both $P < 0.001$) [Figure 4E–4F]. Though the expression of IL6 ($P = 0.119$ and 0.768) did not change significantly in PolyIC and LPS-treated mice, the levels of IL-1β (both $P < 0.001$) and MCP-1 (both $P < 0.001$) increased in the serum from PolyIC and LPS-treated mice when compared with the control group [Figure 4G]. H&E images of tumor tissues showed the number of tumor cells in the mice with PolyIC or LPS administration was more than that in the control group [Figure 4H].

### Systemic inflammation affects the polarization of macrophages in the bone marrow

The distribution of immune cells in the bone marrow under condition of systemic inflammation was examined by FCM. The results showed that although the percentage of total MDSCs ($P = 0.852$ and 0.664) did not alter in the bone marrow cells [Figure 5A and 5B], the percentage of G-MDSCs ($P = 0.001$) increased in the group treated with PolyIC and that of M-MDSCs ($P < 0.001$) increased in the group treated with LPS [Figures 5C and 5D]. The percentage of macrophages ($P = 0.008$ and 0.003) was decreased [Figure 5E], but the percentage of M2-like macrophages ($P = 0.023$ and 0.011) was significantly increased [Figure 5F]. In addition, a significantly decreased number of CD4+ T cells ($P < 0.001$) was observed in the mouse group treated with LPS [Figure 5G]. There was no significant difference in the distribution of CD8+ T cells in the PolyIC/LPS-treated mice when compared with the control group ($P = 0.255$ and 0.786). These results suggested that systemic inflammation could affect the polarization of macrophages into M2 type in the bone marrow.

Infiltration of M2 type macrophages supports tumor growth in the condition of systemic inflammation

The distribution of immune cells in the tumor micro-environments was analyzed by FCM. The results showed that total MDSCs ($P = 0.979$ and 0.746), G-MDSCs ($P = 0.230$ and 0.187) and M-MDSCs ($P = 0.591$ and 0.372) did not change statistically in both PolyIC and LPS-induced systemic inflammation groups compared with the control group [Figure 6A–6D]. Total macrophages ($P = 0.006$ and 0.004) were obviously decreased in the tumor tissues in the condition of systemic inflammation, accompanied with decreased percentage of M1 type ($P = 0.017$ and 0.006) and increased percentage of M2 type ($P = 0.002$ and 0.007) [Figure 6E–6F]. Though the distribution of CD4+ T cells ($P = 0.920$ and 0.614) in the Poly IC and LPS-induced groups was not significantly different from that in the control group, the proportion of CD8+ T cells ($P = 0.031$ and 0.023) slightly decreased in the systemic inflammation groups [Figure 6G]. Further more, the expression of TLRs obviously decreased in the mouse treated with PolyIC or LPS [Figure 6H]. These data indicated that LPS/PolyIC-induced systemic inflammation promoted tumor growth, which might be related to the polarization of macrophages into M2 type and decreased expression of TLRs in the tumor tissues.

Discussion

Macrophages are types of pleiotropic cells and are usually divided into two groups. The classic pro-inflammatory M1 macrophages can exert antigen presentation and tumor killing activity by expressing major histocompatibility complex I and II molecules and secreting cytokines such as tumor necrosis factor-α, IL-1β and IL6.[21,22] While M2 type macrophages are involved in the promotion of tissue remodeling and tumor progression, displaying an promoting function of tumor growth.[23,24] The phenotype of macrophages is plastic and regulated by the local tumor microenvironment.[25,26] In solid tumors, macrophages account for the majority of all immune
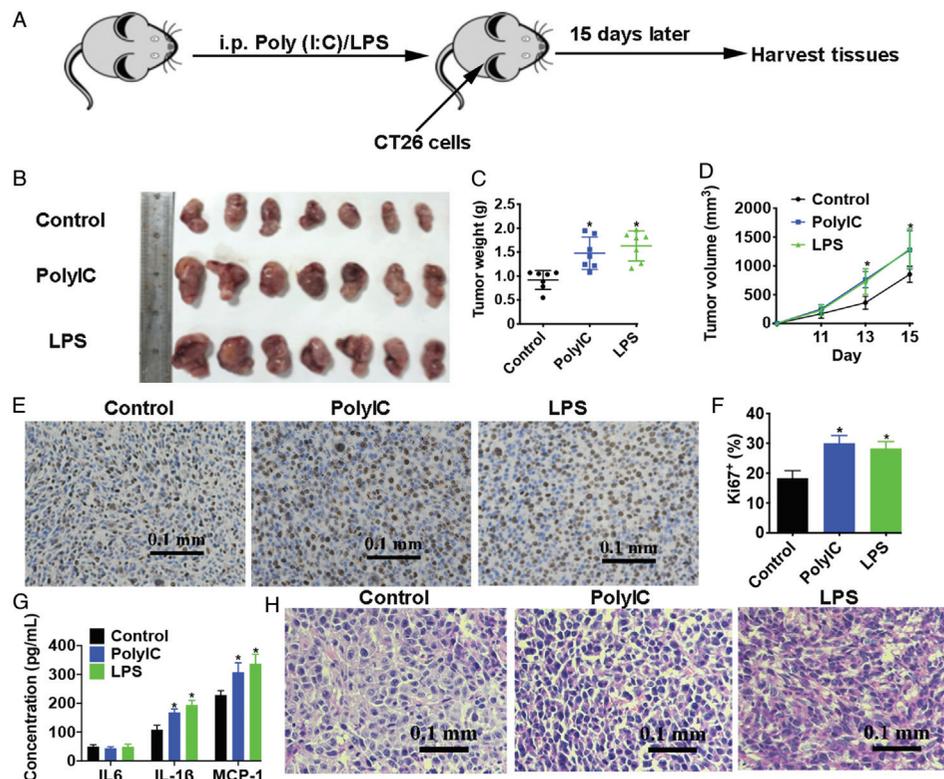
Figure 4: Systemic inflammation induced by intraperitoneal injection of PolyIC or LPS promotes tumor growth. (A) The scheme of mouse model with systemic inflammation. (B–D) Photograph of dissected tumor tissues (B) and quantitative data of tumor weight (C) and volume (D) from mice with the indicated treatment. (E–F) Immunohistochemistry photograph (E) and quantitative data (F) of Ki67 staining of tumor tissues from mice with the indicated treatment. (G) The expression of IL6, IL-1 β, and MCP-1 in the blood serum from mice with the indicated treatment. (H) H&E images of tumor tissues from mice with the indicated treatment. $^{*}P < 0.01$ *vs.* control. H&E: Hematoxylin and eosin; IL: Interleukin; i.p.: Intraperitoneal injection; LPS: Lipopolysaccharides; MCP-1: Monocyte chemoattractant protein-1; Poly (I:C): Polyinosinic acid-polycytidylic acid.

cells.[27] Infiltrating macrophages in tumor microenvironment initially have an M1 phenotype.[28] However, the persistence of various cytokines (such as C-C motif chemokine 2, vascular endothelial growth factor, and macrophage colony-stimulating factor) and hypoxic condition make macrophages polarize into M2-type or recruit circulating monocytes to differentiate into M2-like macrophages, thereby losing phagocytosis and the presentation ability of tumor-associated antigens to T cells.[29,30] In this study, we found local inflammation induced by foreign body implantation significantly suppressed tumor growth with increased infiltration of macrophages especially M1 type in the tumor tissues. In contrast, systemic inflammation induced by intraperitoneal injection of PolyIC or LPS resulted in the reduction of tumoricidal M1 macrophages and the increase of suppressive M2 type, which may mediate its promotion of tumor growth.

Interestingly, the expression of all TLRs changed to varying degrees in the two mouse models. TLRs, one of the pattern recognition receptors, are expressed on the surface of immune cell surface and intracellular vesicles and can recognize pathogen/damage-related molecular patterns.[31,32] Through myeloid differentiation factor 88 or Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF) pathways, TLRs induce the expression of inflammatory cytokines and chemokines thereby regulating immune responses and participating in the inflammation and cancer progression.[33,34] Preivous studies have

reported that TLR3 and TLR4 could facilitate M1 macrophage polarization. TLR3 agonist (LPS) is traditionally used to polarize macrophages into M1 type.[35,36] TLR4 was involved in the activation of nuclear factor-κB signialing, which pariticpated in the M1 polarization of macrophages.[37,38] Some TLR ligands, including TLR2, TLR7/8, and TLR9, can be used for tumor treatment by regulating immune cells.[39] TLR9 ligand CpG strongly inhibited the suppressive function of MDSCs in tumor models.[40] TLR7/8 agonist could promote MDSCs to differentiate into M1-like macrophages.[41] Consistent with this, we found that compared with the control group, the expression of TLRs sharply increased in the local inflammation group, which suggested that activated TLRs may contribute to decreased MDSCs and increased M1-type macrophages in the tumor tissues. However, the expression of TLRs was down-regulated in the systemic inflammation group with the opposite macrophage infiltration. These results suggested the role of inflammation in cancer might depend on the status of TLRs.

In the present study, we found that the foreign body-induced local inflammation inhibited tumor growth, while LPS or PolyIC-induced systemic inflammation promoted tumor growth. The results suggested that the local inflammation-then-cancer and systemic inflammation-then-cancer models might lead to the opposite effects on cancer progression and induce different intra-cancer immune cell infiltration via changing the expression of TLRs.
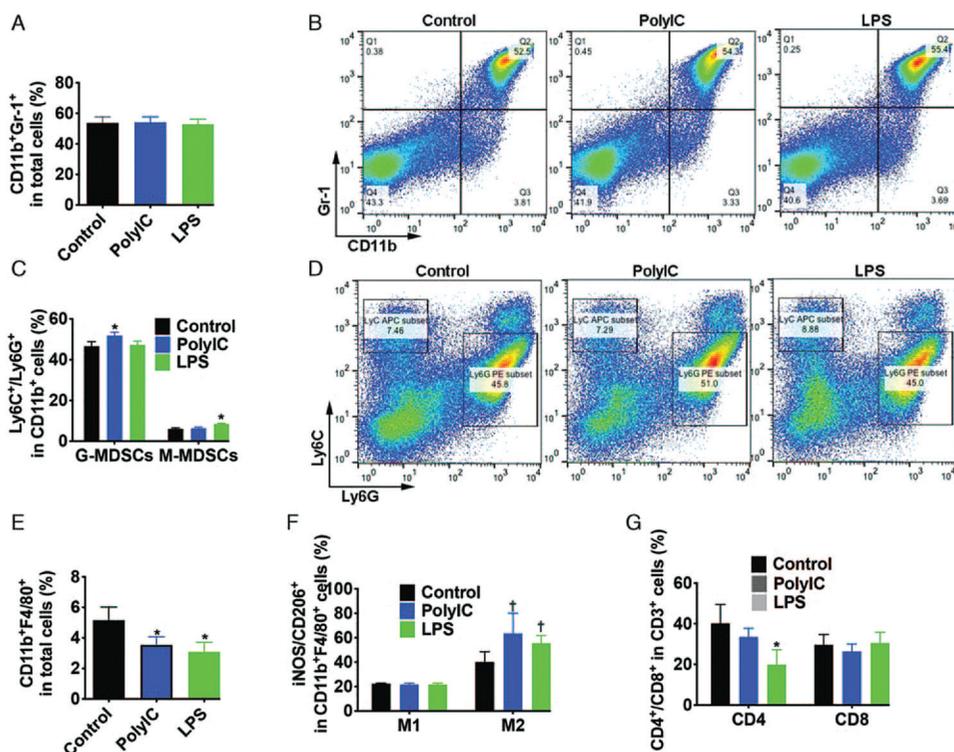
Figure 5: Systemic inflammation affects the polarization of macrophages in bone marrow cells *in vivo*. (A–B) Quantitative data of the proportions (A) and FCM photographs of MDSCs (B) in the bone marrow cells from mice with indicated treatments. (C–D) Quantitative data of the proportions (C) and FCM photographs (D) of subgroups of MDSCs in the bone marrow cells from mice with indicated treatments. (E–G) Quantitative data of the proportions of macrophages (E), subgroups of macrophages (F), and CD4$^+$ and CD8$^+$ T cells (G) in the bone marrow cells from mice with indicated treatments. $^*P < 0.01$, $^\dagger P < 0.05$ *vs.* control. FCM: Flow cytometry; G-MDSCs: Granulocyte-myeloid-derived suppressor cells; Gr-1: Glutathione reductase-1; iNOS: Inducible nitric oxide synthase; LPS: Lipopolysaccharides; M-MDSCs: Monocytic-myeloid-derived suppressor cells.
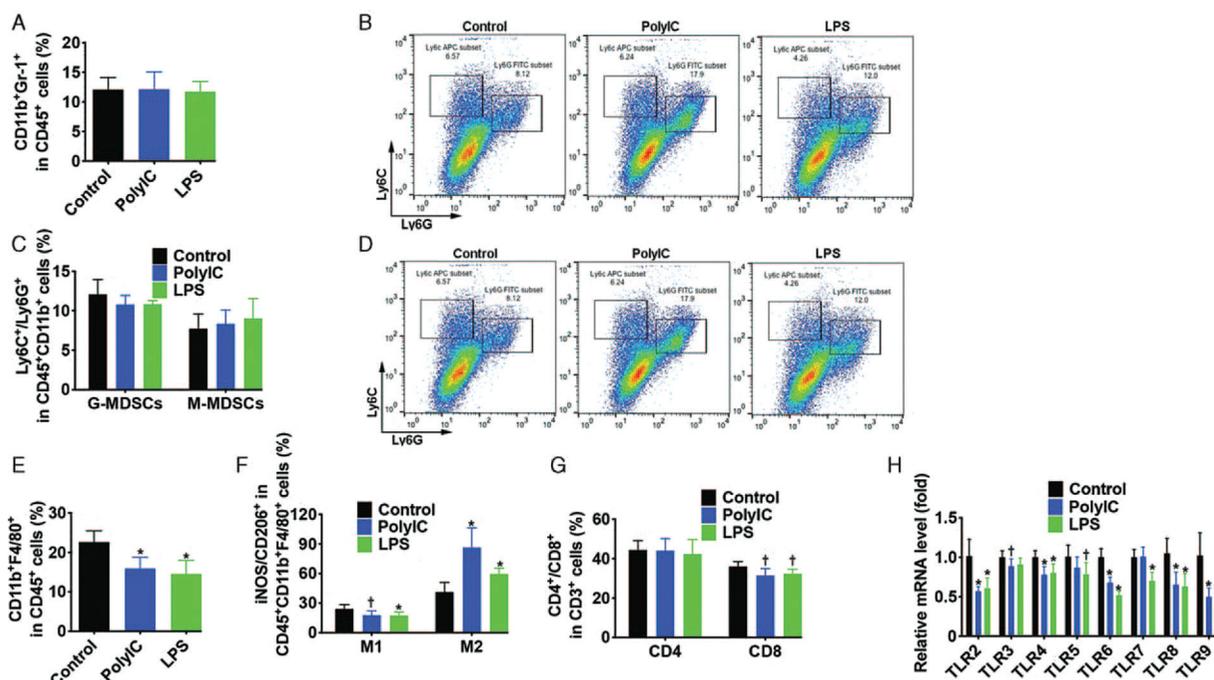
Figure 6: Systemic inflammation enhances infiltration of M2 macrophages into tumor microenvironment *in vivo*. (A–B) Quantitative data of the proportions (A) and FCM photographs (B) of MDSCs in the tumor tissues from mice with indicated treatments. (C–D) Quantitative data of the proportions (C) and FCM photographs (D) of subgroups of MDSCs in the tumor tissues from mice with indicated treatments. (E–G) Quantitative data of the proportions of macrophages (E), subgroups of macrophages (F), and CD4$^+$ and CD8$^+$ T cells (G) in the tumor tissues from mice with indicated treatments. (H) Single cells were extracted from the tumor tissues and used for qRT-PCR to determine the expression of targeted genes. $^*P < 0.01$, $^\dagger P < 0.05$ *vs.* control. FCM: Flow cytometry; G-MDSCs: Granulocyte-myeloid-derived suppressor cells; Gr-1: Glutathione reductase-1; iNOS: Inducible nitric oxide synthase; LPS: Lipopolysaccharides; M-MDSCs: Monocytic-myeloid-derived suppressor cells; qRT-PCR: Quantitative real time-polymerase chain reaction; TLR: Toll-like receptor.

Conflicts of interest

None.

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