Intraperitoneal oxaliplatin administration inhibits the tumor immunosuppressive microenvironment in an abdominal implantation model of colon cancer

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Abstract. Recent studies have demonstrated that some chemotherapeutic drugs can enhance antitumor immunity by eliminating and inactivating immunosuppressive cells. Oxaliplatin (OXP) induces immunogenic cell death by increasing the immunogenicity of cancer cells. However, the effects of OXP on the tumor immunosuppressive microenvironment remain unclear. The aim of the present study was to evaluate the antitumor activity of OXP by intraperitoneal (i.p.) administration in an abdominal implantation model of colon cancer and tested the tumor immune microenvironment to observe whether OXP affects the local immune inhibitory cell populations. Abdominal metastasis models were established by inoculation of CT26 cells. The antitumor efficacy of OXP and the tumor immune microenvironment were evaluated. The tumors and spleens of mice were harvested for flow cytometric analysis. Cluster of differentiation (CD)-8+CD69+ T cells, regulatory T cells (Tregs), CD11b+F4/80^{high} macrophages and myeloid-derived suppressor cells (MDSCs) were evaluated by flow cytometric analysis. *In vivo* i.p. administration of OXP inhibited tumor growth in the abdominal metastasis model. Furthermore, OXP was observed to increase tumor-infiltrating activated CD8+ T cells in tumors, decrease CD11b+F4/80^{high} macrophages in tumors and decrease MDSCs in the spleen. These results suggested that i.p. administration of OXP alone may inhibit tumor cell growth and induce the antitumor immunostimulatory microenvironment by eliminating immunosuppressive cells.

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Introduction

Chemotherapy continues to be the most widely employed therapeutic option for cancer treatment. Chemotherapeutic drugs were previously assumed to suppress body's immune system and detrimental to the efficacy of immunotherapy because of their nonspecific cytostatic and cytotoxic effects. However, an increasing number of studies have reported that some cancer chemotherapeutic drugs stimulate anticancer immune responses under certain conditions. Doxorubicin, cyclophosphamide, gemcitabine, and paclitaxel have been proved to induce immunogenic cell death (ICD) in cancer through surface exposure of calreticulin and release of adenosine triphosphate (ATP) and high-mobility group protein box-1 (HMGB-1) (1-7).

Although ICD is the most important way to trigger a chemotherapy-induced immune response against cancer, it is not the only way (8,9). Recent studies have demonstrated that some chemotherapeutic drugs and agents can enhance antitumor immunity by eliminating and inactivating immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), T regulatory cells (Tregs), and tumor-associated macrophages (TAMs) (10-17). Paradoxically, some chemotherapy agents, such as cyclophosphamide, melphalan and doxorubicin, have been shown to induce MDSCs or TAMs that inhibit immune responses (18-20).

Oxaliplatin (OXP) is effective against many solid tumors and is commonly used in the treatment of colorectal cancer, gastric cancer, pancreatic cancer and ovarian cancer. The major antitumor mechanism is the induction of tumor cell apoptosis by inhibition of DNA synthesis through their covalent binding to DNA in cells (21). Apart from its the cytotoxic properties, recent studies have shown that OXP has the ability to increase the immunogenicity of cancer cells and induce ICD (22). The effects of OXP on the tumor immunosuppressive microenvironment are not clearly understood, and the cellular identity of OXP-induced suppressor cells has not been well studied.

Peritoneal metastasis occurs on 40% of patients with colorectal cancer. It is one of the most common metastasis pathways and compromises the long-term survival of patients with colorectal cancer. OXP is a primarily used chemotherapeutic

agent in the intraperitoneal (i.p.) chemotherapy for peritoneal metastasis in colorectal cancer patients (23,24). In our previous study, we have reported the antitumor effect of OXP plus IL-7 and showed that combined treatment inhibits tumor cell growth partly by immunoregulation. In that study, we found that OXP alone did not show antitumor effects or induce any anti-immune reactions when intravenously administered for the treatment of lung metastasis of colon cancer. However, in the abdominal implantation model, OXP was intraperitoneally administered and significant antitumor effect was observed. Interestingly, Buhtoiarov *et al* (25) found that i.p. and intravenous (i.v.) chemotherapy regimens induce different antitumor effects and immune reactions. After reviewing the relevant studies, we found that there had been no research on changes in the tumor immune microenvironment when i.p. OXP administration was performed to treat peritoneal metastasis of colon cancer. We believed that the tumor immune microenvironment in the peritoneal metastasis model might be exposed to a very high concentration of OXP following i.p. administration and might affect immune cells. Thus, in the current study, we evaluated the antitumor activity and changes in the tumor immune microenvironment following OXP administration in the abdominal implantation model.

Materials and methods

Cell line. The murine CT26 colon carcinoma line was purchased from American Type Culture Collection (ATCC). CT26 cells were inoculated in 75 cm^2 culture flasks and maintained in RPMI-1640 media supplemented with 10% FBS and 100 U/ml penicillin.

Mouse model of colon tumorigenesis. Pathogen-free female BALB/c mice were obtained from Vital River Laboratory Animal Technology Co., Ltd., Beijing and used at the age of 6-8 weeks. The mice were kept under specific pathogen-free conditions stated in the institution guidelines. All experiments were approved by the Animal Ethics Committee of Sichuan University (Sichuan, China). $2x10^5$ CT26 colon cancer cells were injected into peritoneal cavities to build an abdominal metastasis model. Five days after tumor inoculation, the mice were randomized into control group and an OXP-treated group, each with ten mice. Six of each group were used to detect the number and weight of tumor nodules. Three of each group were used to do flow cytometric analysis. Further, 5 mg/kg OXP or phosphate buffer solution (PBS) was intraperitoneally injected on days 6, 9, 12, 15 after tumor inoculation. Thirteen days after the first treatment, the animals were euthanized and tumor nodules in the peritoneal cavity were counted and weighed.

Reagents. OXP was purchased from Sanofi S.A. (Paris, France). Fluorescent-conjugated flow cytometry anti-mouse antibodies (Abs) cluster of differentiation (CD)8 (cat. no. 553030; 1:100), CD69 (cat. no. 551113; 1:100), CD11b (cat. no. 553312; 1:100), Gr-1 (cat. no. 551460; 1:100) for flow cytometry were purchased from BD Pharmingen. Antibody F4/80 (cat. no. 123110; 1:100; BD Biosciences, Franklin Lakes, NJ, USA) for flow cytometry was purchased from BioLegend, Inc. (San Diego, CA, USA). The mouse regulatory T cell staining kit (cat. no. 88-8118-40; 1:100) for flow cytometry was obtained from eBioscience.

Flow cytometric analysis. The tumor cells and spleen cells were harvested from the mice. Tumor cells were minced and digested in a cocktail containing collagenase i.v. (1 mg/ml) and DNase (0.1 mg/ml) in RPMI-1640 for 1 h at 37˚C. Osmotic lysis was used to remove the erythrocytes from the spleen cells. Next, single-cell suspensions of tumor cells and spleen cells were stained using fluorochrome-labeled antibodies, CD4, CD8, CD69, CD11b, F4/80, and matched isotype control antibodies. The mouse regulatory T cell staining kit was used to stain intercellular FoxP3. After staining, the cells were analyzed using the FACSCalibur flow-cytometer (BD Biosciences) and the data were analyzed using the FlowJo software version 7.6 (FlowJo LLC, Ashland, OR, USA).

Statistical analysis. GraphPad Prism version 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) was used to perform statistical analysis and create figures. Results are presented as the mean ± standard error. The unpaired Student's t-test was used for paired comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Inhibitory effect of i.p. OXP administration against abdominal metastasis of colon cancer. To investigate the inhibitory effect of i.p. OXP administration on tumor growth in case of abdominal metastasis in mice, we established the abdominal metastasis model as mentioned above. The mice were randomized into PBS and OXP (5 mg/kg/injection) groups and, each with 10 mice. The tumor nodules of each mouse were cut, counted, and weighed after euthanasia. As shown in Fig. 1 (n=6), i.p. OXP administration significantly reduced the weight (0.04±0.012) g and number (26.00±9.539) of tumor nodules compared with PBS administration (2.350±0.142) g and (267.7±5.897) ($P<0.0001$).

i.p. OXP administration significantly increased activated T cells in the tumors of the abdominal implantation model. Tumor cells were harvested from the OXP and PBS groups and single-cell suspensions were made. Further, activated CD8+ T cells in each group were analyzed by flow cytometry. CD69 is a marker of T-cell activation. We determined whether i.p. OXP administration increases the number of activated CD8+ T cells (CD8+CD69+ population) in the tumors. As shown in Fig. 2 (n=3), i.p. OXP administration significantly increased the number of CD8+CD69+ positive T cells in tumor tissue compared with PBS administration ($P<0.05$). The percent of CD8+CD69+T cells in the two groups were 6.135±0.125 and 4.275±0.135%, respectively.

i.p. OXP administration had no effect on Tregs (CD4+Foxp3+T cells) of spleen in the abdominal implantation model. The fork-head/winged helix transcription factor, FoxP3, is a relatively specific marker for Treg cells (26). The Treg cells in the tumor are very few and flow cytometry is difficult to detect. We detected the tumor for Treg cells and we failed. We evaluated whether i.p. OXP administration had effect on Treg cell populations (CD4+FoxP3+ population) of spleens in the abdominal metastasis mice. Spleens from the two groups of mice were harvested, and the percentage of CD4+FoxP3+

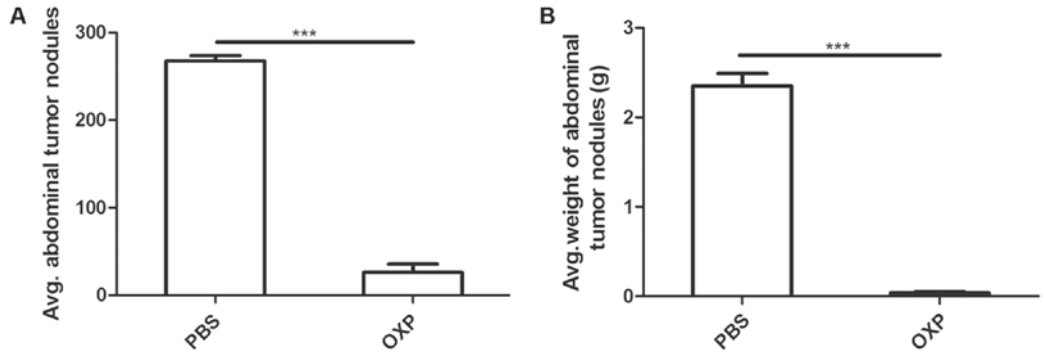

Figure 1. Antitumor effect of i.p. OXP administration in the abdominal metastasis of the colon cancer model in mice. Five days following tumor inoculation, the mice were randomized into the control and OXP-treated groups (n=10/group). A total of 13 days following the first treatment, the mice were euthanized and the nodules in the peritoneal cavity were counted and weighed. (A) The number of tumor nodules in the abdomen metastasis model. (B) Weight of tumor nodules in the abdominal metastasis model. i.p. OXP administration resulted in a significant reduction in the weight and number of tumor nodules in the abdominal cavity when compared with PBS administration. The results are expressed as mean ± standard error (n=6). ***P<0.001, as indicated. i.p., intraperitoneal; OXP, oxaliplatin; PBS, phosphate buffer solution.

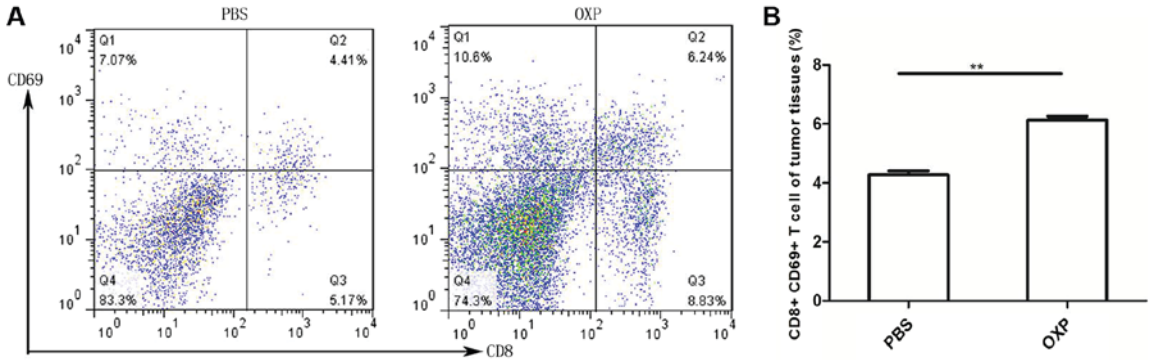

Figure 2. i.p. OXP administration induces significant T cell activation in tumors. (A) Representative flow cytometry data of each group. (B) CD8+/CD69+ positive cells were significantly increased in the OXP group when compared with the control group. The results are expressed as the mean ± standard error (n=3). **P<0.01, as indicated. i.p., intraperitoneal; OXP, oxaliplatin; CD, cluster of differentiation.

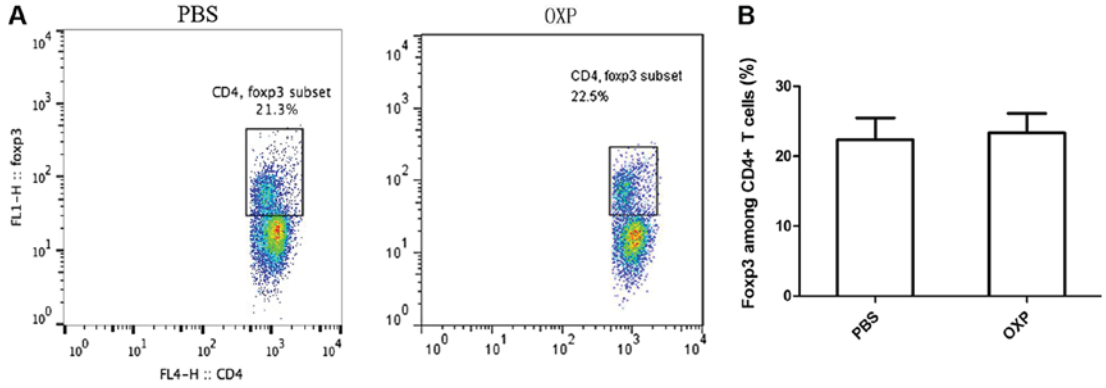

Figure 3. OXP had no effect on Treg cells of the spleen. (A) Typical flow cytometry data of each group are presented. (B) OXP alone had no detectable effect on the FoxP3+ population in CD4+ T cells from the spleen when compared with the control group. The results are expressed as the mean ± standard error (n=3). i.p., intraperitoneal; OXP, oxaliplatin; CD, cluster of differentiation; FoxP3, forkhead box P3; PBS, phosphate buffer solution.

population was analyzed. The percentages of Tregs in spleens was 22.37±3.132% in the group with i.p. administration and 23.37±2.748% in the control group with PBS administration, which was not a significant difference (P>0.05) (Fig. 3) (n=3).

i.p. OXP administration reduced TAMs (CD11b+F4/80^{high}) of the tumor in the abdominal implantation mode. We examined the effects of i.p. administration on the TAMs (CD11b+ F4/80^{high}) (27). Single-cell suspensions of tumor tissue from the OXP group and PBS group were harvested and analyzed for CD11b+F4/80^{high} macrophages cells by flow cytometry. As shown in Fig. 4 (n=3), OXP administration significantly decreased the number of CD11b+F4/80^{high} cells in tumor tissue compared with PBS administration (P<0.01). The percentages

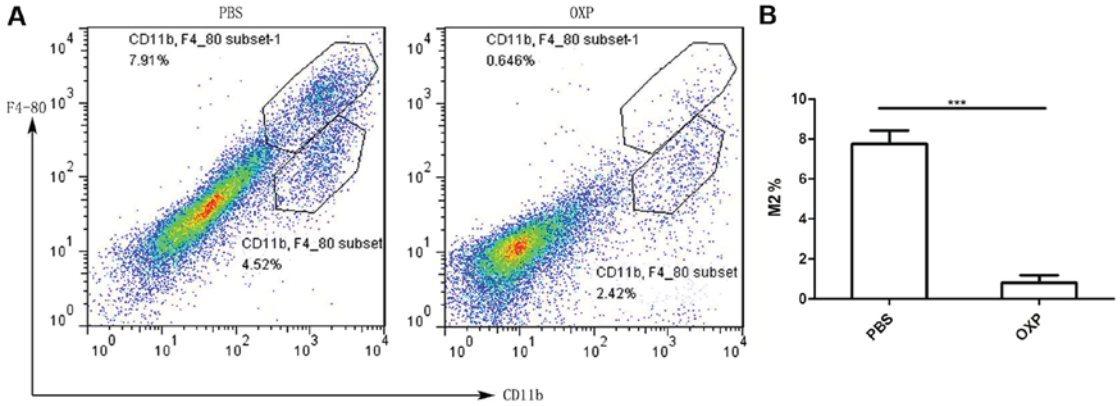

Figure 4. OXP reduces the number of CD11b+F4/80^{high} cells (tumor-associated macrophages) in the tumor microenvironment. (A) Typical flow cytometry data of each group are presented. (B) i.p. OXP administration alone reduced the number of CD11b+F4/80^{high} cells in the tumor microenvironment when compared with the control group in the abdominal metastasis model. The results are expressed as the mean ± standard error (n=3). ***P<0.001, as indicated. i.p., intraperitoneal; OXP, oxaliplatin; CD, cluster of differentiation.

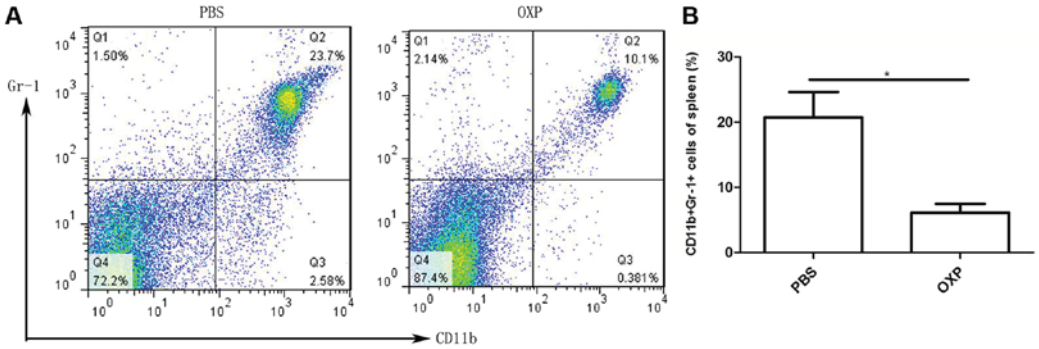

Figure 5. OXP alone reduces the number of CD11b+Gr-1+ cells (myeloid-derived suppressor cells) in the spleen microenvironment. (A) Typical flow cytometry data of each group are presented. (B) OXP alone reduced the number of CD11b+Gr-1+ cells in the spleen microenvironment compared with the control group in the abdominal metastasis model. The results are expressed as the mean ± standard error (n=3). *P<0.05, as indicated. i.p., intraperitoneal; OXP, oxaliplatin; CD, cluster of differentiation.

of CD11b+F4/80^{high} cells in the OXP and control groups were 0.797±0.383 and 7.743±0.69%, respectively.

i.p. OXP administration reduced MDSCs (CD11b+Gr-1+ cells) of the spleen in the abdominal implantation model. Spleens from the two groups of mice were collected, and the percentage of MDSCs was detected using the CD11b and Gr-1 antibodies by flow cytometry. The percentage of MDSCs in spleens was 6.12±1.344% in the group with i.p. OXP administration and 20.73±3.897% in the control group with PBS administration. Thus, i.p. OXP administration significantly decreased the number of MDSCs in spleens compared with the control in the abdominal metastasis model (P<0.05) (Fig. 5) (n=3).

Discussion

In vivo i.p. administration of OXP inhibited the growth of tumors in the abdominal metastasis mouse model. Specifically, OXP treatment significantly increased the number of tumor-infiltrating activated CD8+ T cells, decreased the number of CD11b+F4/80^{high} macrophage in tumors, and decreased the number of MDSCs in spleens. These results suggest that i.p. OXP administration might weaken the tumor immune inhibitory microenvironment.

In the tumor microenvironment, tumor cells can promote immunoinhibitory pathways. This is the main reason by virtue of which tumor cells can escape from the host immune system and is impediment of antitumor immunotherapy (28-31). Tregs, MDSCs, immature dendritic cells (DC), and alternatively activated macrophages (M2) are the main immunosuppressive cells (28,32,33). It is one of the most important aims of cancer research to alter the immunosuppressive tumor microenvironment by eliminating and/or inactivating these immunosuppressive cells.

The cytotoxicity of chemotherapeutic agents is nonspecific and can decrease lymphocytes for a short time. Short-term lymphocytopenia may be beneficial for cancer patients (34-36). Lympho-depletion results in a decrease in the number of immunosuppressive cells, such as Tregs and MDSCs. Recent studies have demonstrated that, apart from their direct cytotoxic effects, several cytotoxic chemotherapeutic drugs may block immunoinhibitory signal networks and enhance antitumor immunity (31).

MDSCs, a heterogeneous population of undifferentiated myeloid cells, accumulate in the tumor microenvironment (37). MDSCs are the main immunosuppressive cells in tumors, including those of colorectal cancer (38,39). It has been demonstrated that some anticancer agents can enhance anticancer

immunity by eliminating or inhibiting MDSCs. Gemcitabine combined with 5-fluorouracil (5FU) has been reported to induce MDSCs apoptosis and deplete MDSCs (15). It has also been shown that Taxanes (docetaxel, paclitaxel) can impair the function of MDSCs and promote MDSCs differentiation into dendritic cells (16,40). Doxorubicin was also observed to decrease MDSCs in the spleen and tumor tissue and weaken the inhibitory function of residual MDSCs (11,41). These chemotherapeutic agents may inhibit MDSCs by different mechanisms. 5FU may selectively kill MDSCs because the expression of thymidylate synthase in MDSCs is lower (15). Docetaxel can block Stat3 phosphorylation and then induce development of M1 macrophages (16). Doxorubicin may eliminate MDSCs because the proliferation status of MDSCs is higher than that of T cells or NK cells (11). In our study, we demonstrated that i.p. OXP administration decreased MDSCs in the spleen in the abdominal metastasis model. This effect has been previously observed in patients with advanced colorectal cancer (19). FOLFOX (folinic acid, 5FU, and OXP) and FOLFIRI (folinic acid, 5FU, and irinotecan) are the commonly used treatment regimens for advanced colorectal cancer. Kanterman *et al* (19) revealed that FOLFOX and FOLFIRI have opposite effects on MDSCs and immune status in patients with advanced colorectal cancer. For example, FOLFOX reduced the accumulation of MDSCs in blood, whereas FOLFIRI enhanced the suppressive environment (19).

TAMs are activated by cancer cells. Studies have shown that the proportion of TAMs is as high as 60% in the tumor stroma (42). Most of TAMs in tumor microenvironments are of the M2 phenotype. Macrophages can be phenotypically polarized by the microenvironment. There are two main polarized groups: classically activated (M1) and alternatively activated (M2) macrophages (10). M1 macrophages are generally considered pro-inflammatory and secrete interleukin-12, oxygen species, nitric oxide and tumor necrosis factor. In contrast, M2 macrophages are considered to have anti-inflammatory effect and induce production of interleukin-10 and transforming growth factor (TGF-β). M1 and M2 macrophages can transform into each other (43) IL-10 and TGF-β, which are produced by various of tumor cells in the tumor microenvironment, induce M2 polarization. TAMs can promote tumor cell growth and metastasis, angiogenesis, adaptive immunity and stroma formation by producing various growth factors (44-46). Previous studies have shown that TAMs are correlated with poor prognosis in a variety of cancers (47,48). Thus, TAMs are now considered as a promising target for anticancer therapy (49-51). However, little research has been conducted on the influence of traditional chemotherapy drugs on TAMs. Buhtoiarov *et al* (25) found by employing a combined chemotherapy regimen (vincristine, cyclophosphamide, and doxorubicin) alone or with immunotherapy, caused the phenotype of TAMs in tumors to change from M2 to M1. In contrast, Dijkgraaf *et al* (20) found that cisplatin or carboplatin *in vitro* induce M2 macrophages in tumor cell lines. The peritoneal cavity normally harbors naïve macrophages that play essential roles in regulating tissue repair and inflammatory responses. Cancer cells can polarize peritoneal macrophages toward an M2 phenotype. M2 macrophages were found to be increased in malignant ascites in xenograft models (52-54). Depletion of macrophages was shown to block ovarian tumor progression and inhibit tumor-associated ascites *in vivo* (55). In our study, i.p. administration of OXP *in vivo* inhibited peritoneal tumor growth and ascites accumulation in the abdominal metastasis model and markedly decreased TAMs in tumors. It suggests that i.p. OXP might decreased TAMs in the tumor microenvironment and induce antitumor effects.

In addition, Tregs, another type of immunosuppressive cell, can control anticancer immune response (56). Cyclophosphamide, paclitaxel, and temozolomide at low-doses have been reported to selectively reduce the number of Tregs and improve the effect of immunotherapy (14,57,58). However, in our study, i.p. OXP did not show any effect on the proportion of Tregs compared with control.

Utilizing chemotherapeutic agents to interfere with immunosuppressive cells is an appealing strategy. Our study showed that i.p. OXP administration may transform the immunosuppressive microenvironment into an immuno-stimulatory microenvironment. These findings would be useful to design more effective chemoimmunotherapeutic strategies. In fact, OXP combined with immunotherapy has demonstrated synergistic antitumor effects in previous research. For example, Gonzalez-Aparicio *et al* (59) found that OXP combined with liver-specific expression of interleukin-12 reduces the immunosuppressive microenvironment and inhibited tumor metastasis in colorectal cancer mice models. In our previous study, we also found that OXP plus interleukin-7 inhibited tumor growth accompanying significant infiltration of activated T cells in the tumor microenvironment (60).

In our previous study, we found that i.v. administration of OXP alone exerted no antitumor effect and had no effect on the number of TAMs (CD11b+F4/80high cells) in the lung metastasis model of colon cancer. In this study, we found that i.p. administration of OXP alone inhibits the growth of tumor and decreased the number of TAMs and MDSCs in abdominal metastasis model of colon cancer. Immune reactions induced by OXP differ with the different routes of administration. We speculated that i.p. OXP administration resulted in very high concentrations of OXP in the tumor environment in the peritoneal metastasis model; hence, different immune reactions were induced as compared with i.v. OXP administration. Our results suggest the presence of an unrecognized underlying mechanism when OXP is administrated via intraepithelial route; however, further studies are required to obtain the details of the mechanisms of immuno-stimulatory properties of OXP.

In the current, we showed that i.p. OXP administration may induce an immune response against tumors by weakening the immunosuppressive microenvironment in abdominal metastasis of colon cancer in mice. These findings may aid in the designing of more effective chemoimmunotherapeutic strategies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

X-CC and H-FG designed the present study, and analyzed and interpreted the data. H-FG performed flow cytometry and was a major contributor in writing the manuscript. LZ and JH performed the animal experiments and flow cytometry. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were approved by the Animal Ethics Committee of Sichuan University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, Schmitt E, Hamai A, Hervas-Stubbs S, Obeid M, *et al*: Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. J Exp Med 202: 1691-1701, 2005.
2. Galluzzi L, Senovilla L, Zitvogel L and Kroemer G: The secret ally: Immunostimulation by anticancer drugs. Nat Rev Drug Discov 11: 215-233, 2012.
3. Hernandez-Alcoceba R and Berraondo P: Immunochemotherapy against colon cancer by gene transfer of interleukin-12 in combination with oxaliplatin. Oncoimmunology 1: 97-99, 2012.
4. Zitvogel L and Kroemer G: Anticancer immunochemotherapy using adjuvants with direct cytotoxic effects. J Clin Invest 119: 2127-2130, 2009.
5. Lake RA and Robinson BW: Immunotherapy and chemotherapy-a practical partnership. Nat Rev Cancer 5: 397-405, 2005.
6. Nowak AK, Robinson BW and Lake RA: Gemcitabine exerts a selective effect on the humoral immune response: Implications for combination chemo-immunotherapy. Cancer Res 62: 2353-2358, 2002.
7. Green DR, Ferguson T, Zitvogel L and Kroemer G: Immunogenic and tolerogenic cell death. Nat Rev Immunol 9: 353-363, 2009.
8. Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, Dawson TM, Dawson VL, El-Deiry WS, Fulda S, *et al*: Molecular definitions of cell death subroutines: Recommendations of the nomenclature committee on cell death 2012. Cell Death Differ 19: 107-120, 2012.
9. Zitvogel L, Galluzzi L, Smyth MJ and Kroemer G: Mechanism of action of conventional and targeted anticancer therapies: Reinstating immunosurveillance. Immunity 39: 74-88, 2013.
10. Mosser DM and Edwards JP: Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8: 958-969, 2008.
11. Alizadeh D, Trad M, Hanke NT, Larmonier CB, Janikashvili N, Bonnotte B, Katsanis E and Larmonier N: Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. Cancer Res 74: 104-118, 2014.
12. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J and Sabzevari H: Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood 105: 2862-2868, 2005.
13. Madajewicz S, Waterhouse DM, Ritch PS, Khan MQ, Higby DJ, Leichman CG, Malik SK, Hentschel P, Gill JF, Zhao L and Nicol SJ: Multicenter, randomized phase II trial of bevacizumab plus folinic acid, fluorouracil, gemcitabine (FFG) versus bevacizumab plus folinic acid, fluorouracil, oxaliplatin (FOLFOX4) as first-line therapy for patients with advanced colorectal cancer. Invest New Drugs 30: 772-778, 2012.
14. Zhang L, Dermawan K, Jin M, Liu R, Zheng H, Xu L, Zhang Y, Cai Y, Chu Y and Xiong S: Differential impairment of regulatory T cells rather than effector T cells by paclitaxel-based chemotherapy. Clin Immunol 129: 219-229, 2008.
15. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, Martin F, Apetoh L, Rébé C and Ghiringhelli F: 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 70: 3052-3061, 2010.
16. Kodumudi KN, Woan K, Gilvary DL, Sahakian E, Wei S and Djeu JY: A novel chemoimmunomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers. Clin Cancer Res 16: 4583-4594, 2010.
17. Suzuki E, Kapoor V, Jassar AS, Kaiser LR and Albelda SM: Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clin Cancer Res 11: 6713-6721, 2005.
18. Ding ZC, Lu X, Yu M, Lemos H, Huang L, Chandler P, Liu K, Walters M, Krasinski A, Mack M, *et al*: Immunosuppressive myeloid cells induced by chemotherapy attenuate antitumor CD4+ T-cell responses through the PD-1-PD-L1 axis. Cancer Res 74: 3441-3453, 2014.
19. Kanterman J, Sade-Feldman M, Biton M, Ish-Shalom E, Lasry A, Goldshtein A, Hubert A and Baniyash M: Adverse immunoregulatory effects of 5FU and CPT11 chemotherapy on myeloid-derived suppressor cells and colorectal cancer outcomes. Cancer Res 74: 6022-6035, 2014.
20. Dijkgraaf EM, Heusinkveld M, Tummers B, Vogelpoel LT, Goedemans R, Jha V, Nortier JW, Welters MJ, Kroep JR and van der Burg SH: Chemotherapy alters monocyte differentiation to favor generation of cancer-supporting M2 macrophages in the tumor microenvironment. Cancer Res 73: 2480-2492, 2013.
21. Graham J, Mushin M and Kirkpatrick P: Oxaliplatin. Nat Rev Drug Discov 3: 11-12, 2004.
22. Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, Aymeric L, Michaud M, Apetoh L, Barault L, *et al*: Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene 29: 482-491, 2010.
23. Gervais MK, Dubé P, McConnell Y, Drolet P, Mitchell A and Sideris L: Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy with oxaliplatin for peritoneal carcinomatosis arising from colorectal cancer. J Surg Oncol 108: 438-443, 2013.
24. Elias D, Gilly F, Boutitie F, Quenet F, Bereder JM, Mansvelt B, Lorimier G, Dubè P and Glehen O: Peritoneal colorectal carcinomatosis treated with surgery and perioperative intraperitoneal chemotherapy: Retrospective analysis of 523 patients from a multicentric French study. J Clin Oncol 28: 63-68, 2010.
25. Buhtoiarov IN, Sondel PM, Wigginton JM, Buhtoiarova TN, Yanke EM, Mahvi DA and Rakhmilevich AL: Anti-tumour synergy of cytotoxic chemotherapy and anti-CD40 plus CpG-ODN immunotherapy through repolarization of tumour-associated macrophages. Immunology 132: 226-239, 2011.
26. Hori S, Nomura T and Sakaguchi S: Control of regulatory T cell development by the transcription factor Foxp3. Science 299: 1057-1061, 2003.
27. Pucci F, Venneri MA, Biziato D, Nonis A, Moi D, Sica A, Di Serio C, Naldini L and De Palma M: A distinguishing gene signature shared by tumor-infiltrating Tie2-expressing monocytes, blood 'resident' monocytes, and embryonic macrophages suggests common functions and developmental relationships. Blood 114: 901-914, 2009.
28. Gabrilovich DI and Nagaraj S: Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9: 162-174, 2009.
29. Mundy-Bosse BL, Lesinski GB, Jaime-Ramirez AC, Benninger K, Khan M, Kuppusamy P, Guenterberg K, Kondadasula SV, Chaudhury AR, La Perle KM, *et al*: Myeloid-derived suppressor cell inhibition of the IFN response in tumor-bearing mice. Cancer Res 71: 5101-5110, 2011.
30. Wesolowski R, Markowitz J and Carson WE III: Myeloid derived suppressor cells-a new therapeutic target in the treatment of cancer. J Immunother Cancer 1: 10, 2013.

31. Alizadeh D and Larmonier N: Chemotherapeutic targeting of cancer-induced immunosuppressive cells. Cancer Res 74: 2663-2668, 2014.
32. Zitvogel L, Tesniere A and Kroemer G: Cancer despite immunosurveillance: Immunoselection and immunosubversion. Nat Rev Immunol 6: 715-727, 2006.
33. Colombo MP and Piconese S: Regulatory-T-cell inhibition versus depletion: The right choice in cancer immunotherapy. Nat Rev Cancer 7: 880-887, 2007.
34. Restifo NP, Dudley ME and Rosenberg SA: Adoptive immunotherapy for cancer: Harnessing the T cell response. Nat Rev Immunol 12: 269-281, 2012.
35. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, *et al*: Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 298: 850-854, 2002.
36. Wrzesinski C, Paulos CM, Gattinoni L, Palmer DC, Kaiser A, Yu Z, Rosenberg SA and Restifo NP: Hematopoietic stem cells promote the expansion and function of adoptively transferred antitumor CD8 T cells. J Clin Invest 117: 492-501, 2007.
37. Gabrilovich DI, Ostrand-Rosenberg S and Bronte V: Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12: 253-268, 2012.
38. Sun HL, Zhou X, Xue YF, Wang K, Shen YF, Mao JJ, Guo HF and Miao ZN: Increased frequency and clinical significance of myeloid-derived suppressor cells in human colorectal carcinoma. World J Gastroenterol 18: 3303-3309, 2012.
39. Kanterman J, Sade-Feldman M and Baniyash M: New insights into chronic inflammation-induced immunosuppression. Semin Cancer Biol 22: 307-318, 2012.
40. Michels T, Shurin GV, Naiditch H, Sevko A, Umansky V and Shurin MR: Paclitaxel promotes differentiation of myeloid-derived suppressor cells into dendritic cells in vitro in a TLR4-independent manner. J Immunotoxicol 9: 292-300, 2012.
41. Mattarollo SR, Loi S, Duret H, Ma Y, Zitvogel L and Smyth MJ: Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. Cancer Res 71: 4809-4820, 2011.
42. Heusinkveld M and van der Burg SH: Identification and manipulation of tumor associated macrophages in human cancers. J Transl Med 9: 216, 2011.
43. Sica A and Mantovani A: Macrophage plasticity and polarization: In vivo veritas. J Clin Invest 122: 787-795, 2012.
44. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C and Zheng L: Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. J Exp Med 206: 1327-1337, 2009.
45. Galdiero MR, Garlanda C, Jaillon S, Marone G and Mantovani A: Tumor associated macrophages and neutrophils in tumor progression. J Cell Physiol 228: 1404-1412, 2013.
46. Lievense LA, Bezemer K, Aerts JG and Hegmans JP: Tumor-associated macrophages in thoracic malignancies. Lung Cancer 80: 256-262, 2013.
47. Zhang H, Wang X, Shen Z, Xu J, Qin J and Sun Y: Infiltration of diametrically polarized macrophages predicts overall survival of patients with gastric cancer after surgical resection. Gastric Cancer 18: 740-750, 2015.
48. Zhang M, He Y, Sun X, Li Q, Wang W, Zhao A and Di W: A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients. J Ovarian Res 7: 19, 2014.
49. Sica A, Schioppa T, Mantovani A and Allavena P: Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: Potential targets of anti-cancer therapy. Eur J Cancer 42: 717-727, 2006.
50. Tang X, Mo C, Wang Y, Wei D and Xiao H: Anti-tumour strategies aiming to target tumour-associated macrophages. Immunology 138: 93-104, 2013.
51. Nywening TM, Wang-Gillam A, Sanford DE, Belt BA, Panni RZ, Cusworth BM, Toriola AT, Nieman RK, Worley LA, Yano M, *et al*: Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: A single-centre, open-label, dose-finding, non-randomised, phase 1b trial. Lancet Oncol 17: 651-662, 2016.
52. Wang X, Deavers M, Patenia R, Bassett RL Jr, Mueller P, Ma Q, Wang E and Freedman RS: Monocyte/macrophage and T-cell infiltrates in peritoneum of patients with ovarian cancer or benign pelvic disease. J Transl Med 4: 30, 2006.
53. Robinson-Smith TM, Isaacsohn I, Mercer CA, Zhou M, Van Rooijen N, Husseinzadeh N, McFarland-Mancini MM and Drew AF: Macrophages mediate inflammation-enhanced metastasis of ovarian tumors in mice. Cancer Res 67: 5708-5716, 2007.
54. Ko SY, Ladanyi A, Lengyel E and Naora H: Expression of the homeobox gene HOXA9 in ovarian cancer induces peritoneal macrophages to acquire an M2 tumor-promoting phenotype. Am J Pathol 184: 271-281, 2014.
55. Bak SP, Walters JJ, Takeya M, Conejo-Garcia JR and Berwin BL: Scavenger receptor-A-targeted leukocyte depletion inhibits peritoneal ovarian tumor progression. Cancer Res 67: 4783-4789, 2007.
56. Shevach EM: Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity 30: 636-645, 2009.
57. Ghiringhelli F, Larmonier N, Schmitt E, Parcellier A, Cathelin D, Garrido C, Chauffert B, Solary E, Bonnotte B and Martin F: CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. Eur J Immunol 34: 336-344, 2004.
58. Banissi C, Ghiringhelli F, Chen L and Carpentier AF: Treg depletion with a low-dose metronomic temozolomide regimen in a rat glioma model. Cancer Immunol Immunother 58: 1627-1634, 2009.
59. Gonzalez-Aparicio M, Alzuguren P, Mauleon I, Medina-Echeverz J, Hervas-Stubbs S, Mancheno U, Berraondo P, Crettaz J, Gonzalez-Aseguinolaza G, Prieto J and Hernandez-Alcoceba R: Oxaliplatin in combination with liver-specific expression of interleukin 12 reduces the immunosuppressive microenvironment of tumours and eradicates metastatic colorectal cancer in mice. Gut 60: 341-349, 2011.
60. Gou HF, Huang J, Shi HS, Chen XC and Wang YS: Chemo-immunotherapy with oxaliplatin and interleukin-7 inhibits colon cancer metastasis in mice. PLoS One 9: e85789, 2014.