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Report

CpG oligodeoxynucleotides potentiate the antitumor activity of anti-BST2 antibody

Kosuke Hiramatsu,[1,2] Satoshi Serada,[2] Kouji Kobiyama,[3] Satoshi Nakagawa,[1,2] Akiko Morimoto,[1] Shinya Matsuzaki,[1] Yutaka Ueda,[1] Minoru Fujimoto,[2] Kiyoshi Yoshino,[1] Ken J. Ishii,[3] Takayuki Enomoto,[4] Tadashi Kimura[1] and Tetsuji Naka[2]

[1]Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Suita, Japan; [2]Laboratory of Immune Signal, National Institute of Biomedical Innovation, Health and Nutrition, Ibaraki, Japan; [3]Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation, Health and Nutrition, Ibaraki, Japan; [4]Department of Obstetrics and Gynecology, Niigata University Medical School, Niigata, Japan

Key words

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Correspondence

Tetsuji Naka, Laboratory of Immune Signal, National Institute of Biomedical Innovation, Health and Nutrition, 7-6-8 Saitoasagi, Ibaraki City, Osaka 567-0085, Japan.
Tel: +81-72-641-9843; Fax: +81-72-641-9837;
E-mail: tnaka@nibiohn.go.jp

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Numerous monoclonal antibodies (mAb) targeting tumor antigens have recently been developed. Antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) via effector cells such as tumor-infiltrating natural killer (NK) cells and macrophages are often involved in mediating the antitumor activity of mAb. CpG oligodeoxynucleotides (ODN) have a potent antitumor activity and are considered to increase tumor infiltration of NK cells and macrophages. Our group previously reported significant antitumor activity of anti-bone marrow stromal antigen 2 (BST2) mAb against BST2-positive endometrial cancer cells through ADCC. In this study, we evaluated the synergistic antitumor activity of combination therapy with anti-BST-2 mAb and CpG ODN using SCID mice and elucidated the mechanisms underlying this activity. Anti-BST2 mAb and CpG ODN monotherapy had a significant dose-dependent antitumor activity ($P = 0.0135$ and $P = 0.0196$, respectively). Combination therapy with anti-BST2 mAb and CpG ODN had a significant antitumor activity in SCID mice ($P < 0.01$), but not in NOG mice. FACS analysis revealed significantly increased numbers of NK cells and macrophages in tumors treated with a combination of anti-BST2 mAb and CpG ODN and with CpG ODN alone in SCID mice ($P < 0.05$ and $P < 0.01$, respectively). These results suggested that the combination therapy with anti-BST2 mAb and CpG ODN has a significant antitumor activity and induces tumor infiltration of NK cells and macrophages. Combination therapy with CpG ODN and anti-BST2 mAb or other antitumor mAb depending on ADCC may represent a new treatment option for cancer.

Molecularly targeted monoclonal antibodies (mAb) for cancer have demonstrated highly specific inhibition of target molecules, while avoiding severe adverse events compared with cytotoxic agents.[1] Antitumor mAbs, which depend on antibody-dependent cellular cytotoxicity (ADCC) and/or antibody-dependent cellular phagocytosis (ADCP) via immune effector cells such as tumor infiltrating natural killer (NK) cells and macrophages, have been considered to play an important role.[2] In addition, it has been reported that decreased infiltration of NK cells is associated with a worse prognosis.[3] Moreover, cancer-induced immunosuppression of NK cells has been reported in patients with various types of cancers, leading to decreased ADCC.[4] Therefore, enhancing tumor infiltration of NK cells and macrophages and reducing immmunosuppression are important to induce efficient ADCC and ADCP in patients with cancer.

CpG oligodeoxynucleotides (ODN) are potent immunostimulants recognized by Toll-like receptor 9 on dendritic cells and B cells.[5] Ishii *et al.* (2003) report the antitumor activities of CpG ODN.[6] In particular, intra-tumoral (i.t.) injection of CpG ODN has been shown to be superior to systemic administration through the induction of i.t. infiltration of NK cells.[6]

Previously, our group reported that bone marrow stromal antigen 2 (BST2) is a therapeutic target for endometrial cancer and demonstrated a potent activity of anti-BST mAb against BST2-positive endometrial cancer cells through ADCC.[7] BST2 was originally identified as a cell surface membrane and expression levels of BST2 are increased in myeloma,[8] making it a potential target for antibody-based therapies against cancer. Because CpG ODN induce i.t. infiltration of NK cells and macrophages, combination therapy with CpG ODN with molecularly targeted mAb depending on ADCC and/or ADCP may demonstrate superior synergistic antitumor activity.

The aim of the present study was to evaluate the synergistic antitumor activity of anti-BST2 mAb and CpG ODN and to elucidate the underlying mechanisms using a xenograft model of BST2-positive endometrial cancer cells.

Materials and Methods

Cell lines and culture. HEC-88nu cells were obtained from the Japanese Collection of Research Bioresources (JCRB, Osaka, Japan) and maintained in DMEM (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 20% FBS and 1% penicillin–streptomycin (Nacalai Tesque, Kyoto, Japan) at 37°C under a humidified atmosphere with 5% CO_2. All experiments are described in Supplementary Data S1.

Results

Anti-bone marrow stromal antigen 2 monoclonal antibody and CpG oligodeoxynucleotides exhibit significant dose-dependent antitumor activity. To determine the optimum concentrations of anti-BST2 mAb and CpG ODN for combination therapy, we evaluated the individual dose-dependent antitumor activity of anti-BST2 mAb and CpG ODN. For the anti-BST2 mAb group, SCID mice xenografted with tumor cells were treated with i.p. injection of 400 μL of PBS or anti-BST2 mAb (12.5, 50 and 200 μg in 400 μL of PBS/mouse). As shown in Figure 1(a), anti-BST2 mAb exhibited a significant dose-dependent reduction in tumor weight (P = 0.0135) and a dose-dependent trend toward reduced tumor volume (P = 0.0552). In the CpG ODN group, xenografted SCID mice were treated with i.t. injection of PBS or CpG ODN (10, 20 and 40 μg in 10 μL of PBS /mouse). As shown in Figure 1(b), CpG ODN exhibited a significant dose-dependent reduction in tumor volume and tumor weight (P = 0.0319 and P = 0.0196, respectively). These

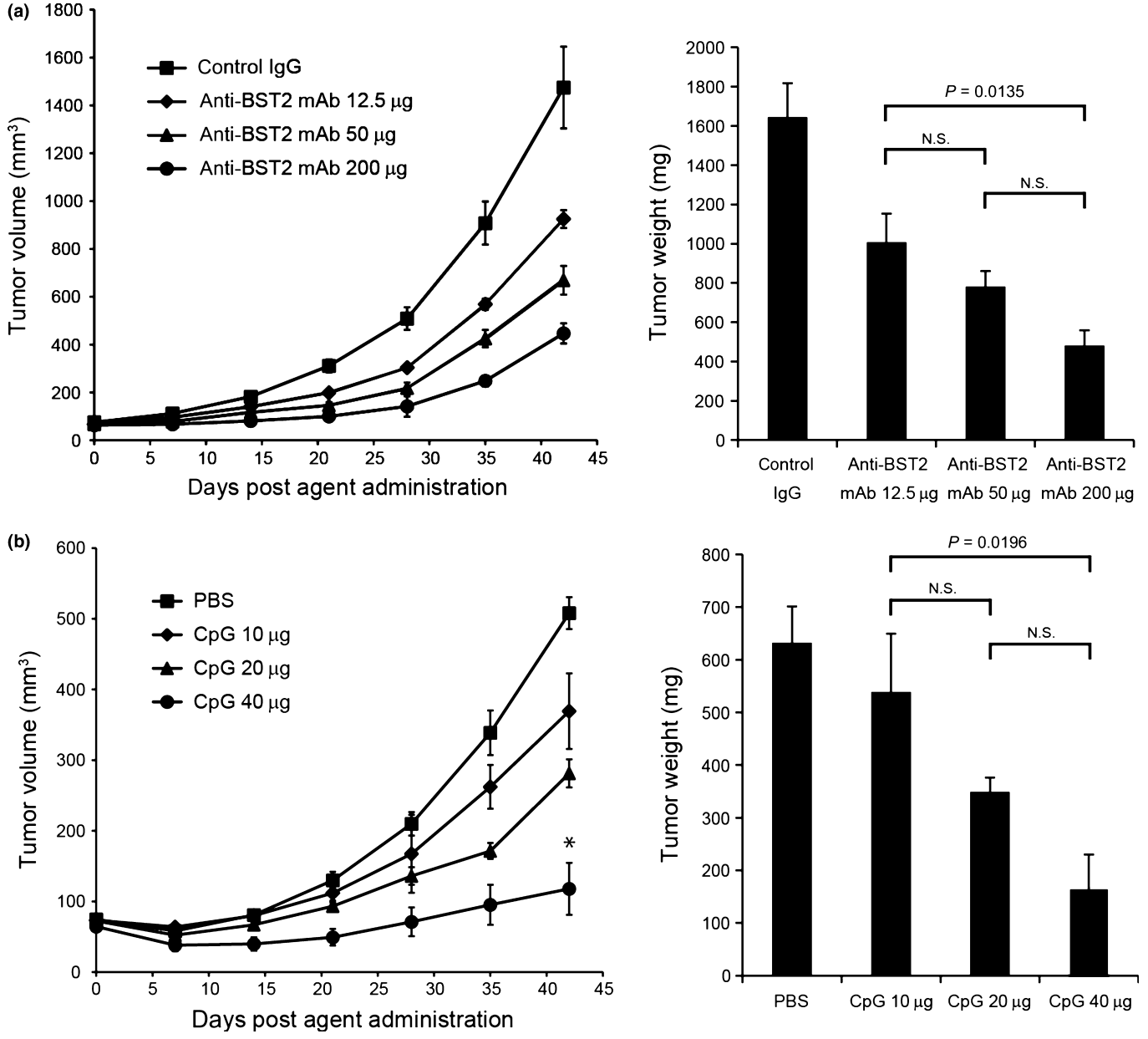

Fig. 1. Anti-bone marrow stromal antigen 2 (BST2) monoclonal antibodies (mAb) and CpG oligodeoxynucleotides (ODN) monotherapy have dose-dependent antitumor activity in SCID mice: (a) xenografted SCID mice treated with 400 μL of PBS or anti-BST2 mAb (12.5, 50 and 200 μg in 400 μL of PBS/mouse) by i.p. injection. Anti-BST2 mAb caused significant dose-dependent decrease in tumor weight (P = 0.0135) and a trend toward reduced tumor volume (P = 0.0552). (b) Xenografted SCID mice treated with PBS or CpG ODN (10, 20 and 40 μg in 10 μL of PBS/mouse) by i.t. injection. CpG ODN caused a significant dose-dependent reduction in tumor volume and weight (P = 0.0319 and P = 0.0196, respectively).

results demonstrate that both anti-BST2 mAb and CpG ODN have a dose-dependent antitumor activities.

Combination therapy with anti-bone marrow stromal antigen 2 monoclonal antibody and CpG oligodeoxynucleotide exhibits synergistic activity in xenografted SCID mice but not in NOG mice. To evaluate the synergistic effect of anti-BST2 mAb and CpG ODN, xenografted SCID mice were treated by i.p./i.t. injection of (A) PBS/PBS, (B) anti-BST2 mAb (12.5 μg/mouse)/PBS, (C) anti-BST2 mAb (200 μg/mouse)/PBS, (D) PBS/CpG ODN (10 μg/mouse) and (E) anti-BST2 mAb (12.5 μg/mouse)/CpG ODN (10 μg/mouse), respectively. In SCID mice, treatment with regimen (e) resulted in a significant antitumor activity compared with other regimens in terms of tumor volume and tumor weight ($P < 0.01$ and $P < 0.05$, respectively; Fig. 2a,b). To reveal whether the synergistic effect of anti-BST2 mAb and CpG ODN is dependent on NK cells, NOG mice that have the complete defect of NK cells and the dysfunction of macrophages were used. In NOG mice xenografted with tumors, all regimes showed no antitumor effects in tumor volume and tumor weight ($P = 1.00$ and $P = 1.00$, respectively; Fig. 2c,d). These results demonstrate a synergistic antitumor effect of combination therapy with anti-BST2 mAb and CpG ODN via NK cells and macrophages.

FACS analysis demonstrates natural killer cell and macrophage infiltration in tumors treated with anti-bone marrow stromal antigen 2 monoclonal antibody and CpG oligodeoxynucleotide. For FACS analysis, xenografted SCID mice were treated by i.p./i.t. injection of (A) PBS/PBS, (B) anti-BST2 mAb (12.5 μg/mouse)/PBS, (C) PBS/CpG ODN (10 μg/mouse) and (D) anti-BST2 mAb (12.5 μg/mouse)/CpG ODN

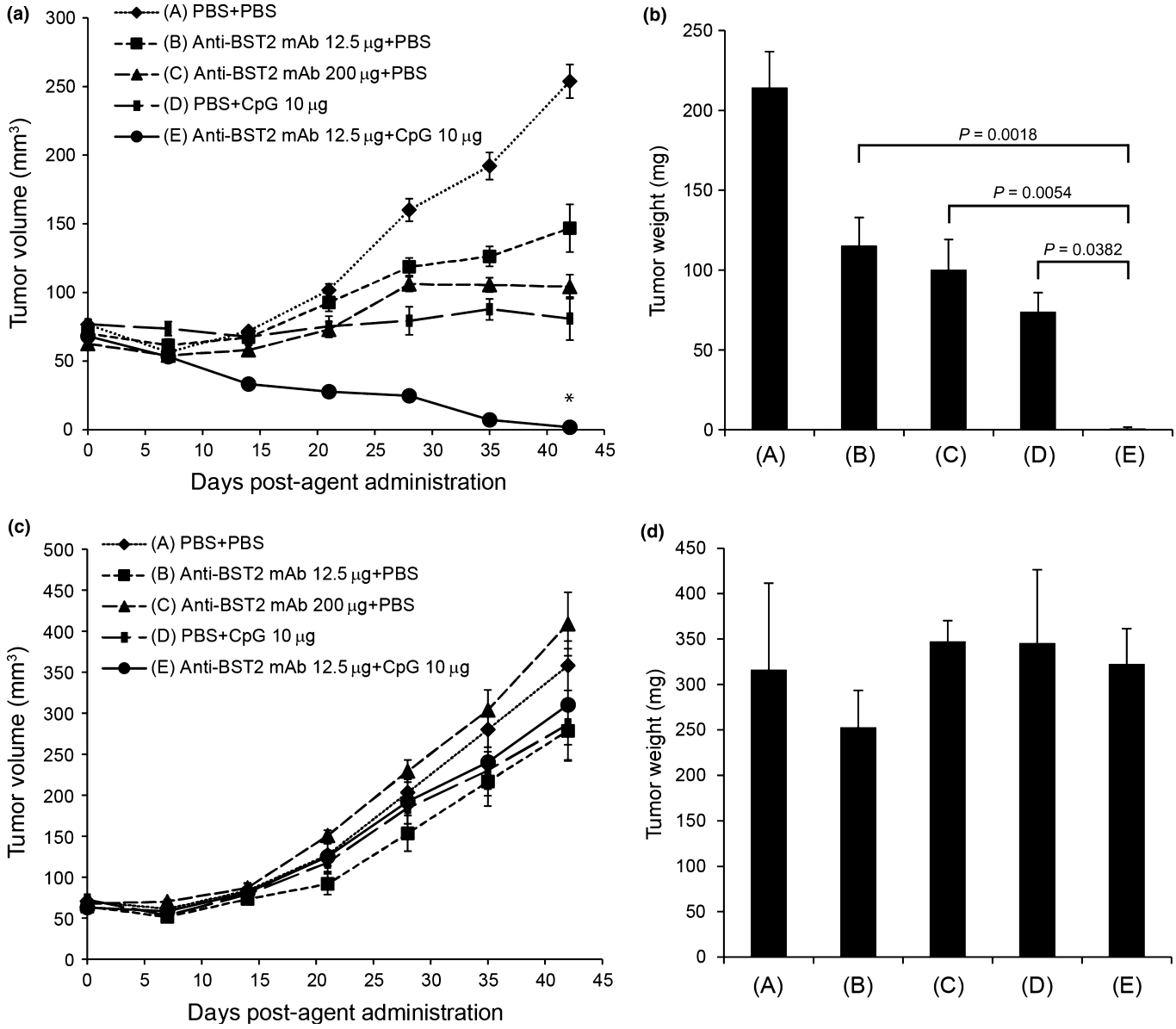

Fig. 2. Combination therapy with anti-bone marrow stromal antigen 2 (BST2) monoclonal antibodies (mAb) and CpG oligodeoxynucleotides (ODN) reveals synergistic antitumor activity. Xenografted SCID (a, b) and NOG (c, d) mice were treated by i.p./i.t. injection of (A) PBS/PBS, (B) anti-BST2 mAb (12.5 μg/mouse)/PBS, (C) anti-BST2 mAb (200 μg/mouse)/PBS, (D) PBS/CpG ODN (10 μg/mouse) and (E) anti-BST2 mAb (12.5 μg/mouse)/CpG ODN (10 μg/mouse), respectively. Treatment with regimen (E) caused a significant decrease in tumor volume compared with that of other regimens in SCID mice ($P < 0.01$).

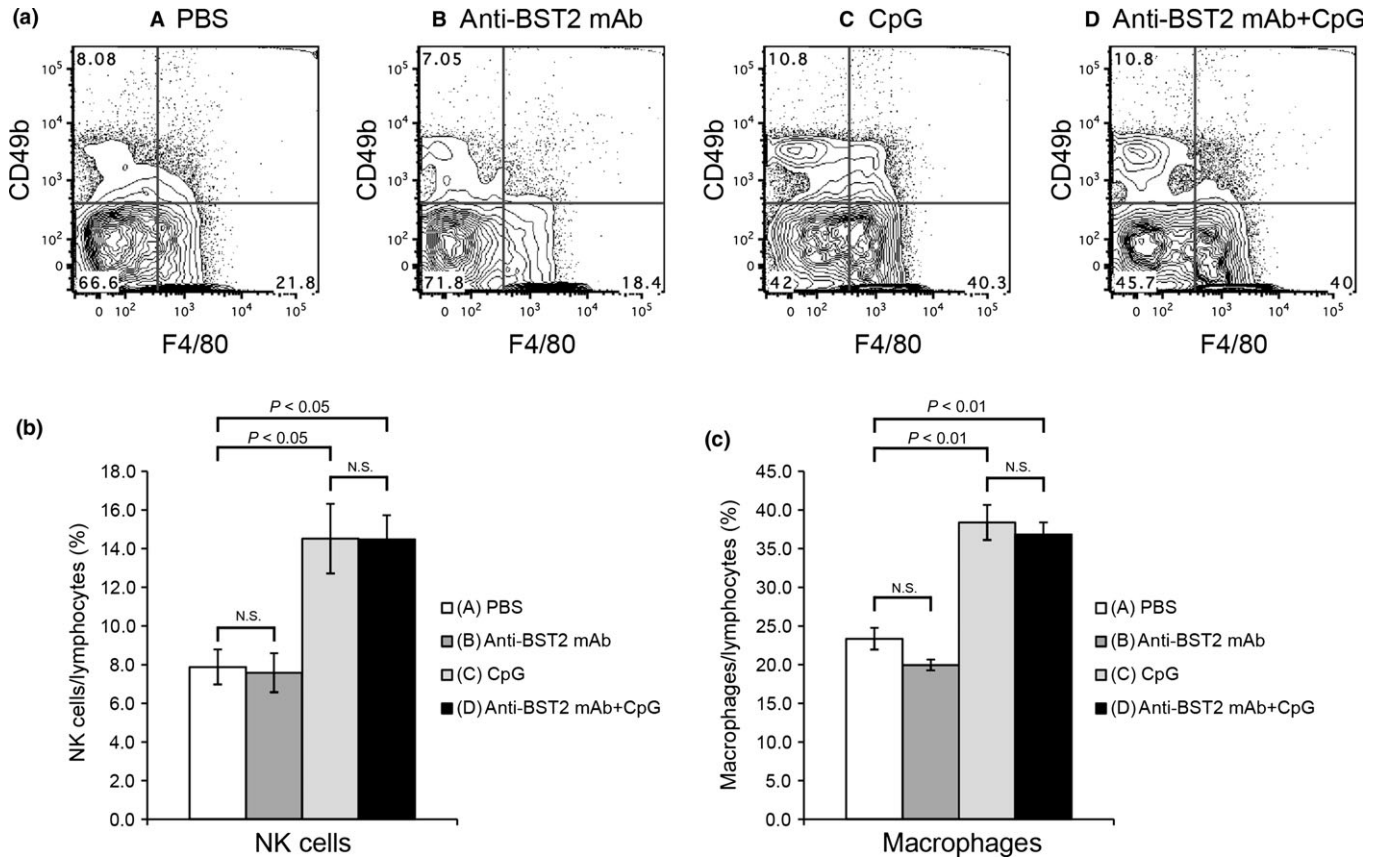

Fig. 3. CpG oligodeoxynucleotides (ODN) increases tumor infiltration of natural killer (NK) cells and macrophages. Xenografted SCID mice were treated by i.p./i.t. injection of (A) PBS/PBS, (B) anti-bone marrow stromal antigen 2 (BST2) monoclonal antibodies (mAb) (12.5 μg/mouse)/PBS, (C) PBS/CpG ODN (10 μg/mouse) or (D) anti-BST2 mAb (12.5 μg/mouse)/CpG ODN (10 μg/mouse). (a) FACS analysis of the tumor-infiltrated macrophages and NK cells. Tumor-infiltrated cells were isolated and gated on $CD45^+$ cells. Representative dot plot data of $F4/80^+$ macrophages and $CD49b^+$ NK cells in tumors treated with regimens (A) to (D) were shown. (b) Significantly higher numbers of NK cells in tumors treated with regimens (C) and (D) than in those treated with regimens (A) and (B) ($P < 0.05$). (c) Significantly higher numbers of macrophages in tumors treated with regimens (C) and (D) than in those treated with regimens (A) and (B) ($P < 0.01$).

(10 μg/mouse), respectively. The dot plots in Figure 3(a) show the representative results of FACS analysis of $F4/80^+$ macrophages and $CD49b^+$ NK cells in tumors treated with regimen (A) to (D).

In tumors from SCID mice treated with regimens (A) and (B), NK cells accounted for 8% of all lymphocytes, whereas the proportion of NK cells was significantly increased in tumors from SCID mice treated with regimens (C) and (D) ($P < 0.05$; Fig. 3b). Similarly, the number of macrophages was significantly higher in tumors from SCID mice treated with regimens (C) and (D) than in those of SCID mice treated with regimens (A) and (B) ($P < 0.01$; Fig. 3c).

Discussion

In this study, we demonstrated a significant therapeutic activity of combination therapy with anti-BST2 mAb and CpG ODN in a BST2-positive endometrial cancer xenograft model. We further demonstrated increased tumor infiltration of NK cells and macrophages in SCID mice treated with CpG ODN.

The preclinical efficacy of combination therapy with CpG ODN and antitumor agents has been reported[9]; however, its antitumor activity has not been fully investigated *in vivo*. In our study, combination therapy with anti-BST2 mAb and CpG ODN also exhibited a potent antitumor activity in a SCID mouse xenograft model. Anti-BST2 mAb exhibit a dose-dependent antitumor effect via ADCC; therefore, greater concentrations of mAb are required to achieve greater efficacy. However, there is concern regarding increased adverse effects with greater doses of anti-BST2 mAb. BST2 is known to be expressed not only on the cell surface of dendritic cells but also in other normal tissues, including spleen, gallbladder and stomach.[10] The anti-BST2 mAb used in the present study recognizes human BST2, but not mouse BST2 (data not shown), suggesting that the humanized form of anti-BST2 mAb may induce cytotoxicity against these cells and tissues. However, CpG ODN increased the activity of anti-BST2 mAb at low doses, suggesting that combination therapy may allow decreased therapeutic doses of anti-BST2 mAb, thereby reducing adverse effects.

The infiltration of NK cells and macrophages plays an important role in inducing ADCC and ADCP. Previously, tumor infiltration of NK cells and macrophages has been analyzed by immunohistochemistry.[11] This is the first report of FACS analysis revealing increased infiltration of NK cells and macrophages using whole-tumor tissues.

Intra-tumoral injection is required for the effective treatment of CpG ODN.[6] In gynecological cancer, i.t. injection is possible for localized cervical cancer, endometrial cancer and recurrent tumor of the vaginal stump, allowing the use of CpG ODN in gynecological cancer. Our study specifically demonstrated the efficacy of combination therapy

with anti-BST2 mAb and CpG ODN; however, CpG ODN could be combined with gynecological antitumor antibodies routinely used in clinical practice. Moreover, in recent studies, a systemically administered CpG ODN-conjugated antitumor antibody demonstrated antitumor activity,[12] suggesting that CpG ODN could also be used in gynecological cancers where deep location prevents direct administration.

In summary, combination therapy with anti-BST2 mAb and CpG ODN exerts significant antitumor activity and induces infiltration of NK cells and macrophages in CpG ODN-treated tumors. Combination therapy with CpG ODN and anti-BST2 mAb or other antitumor agents depending on ADCC may represent a novel treatment option for cancer.

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Disclosure Statement

The authors have no conflict of interest to declare.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Data S1. Supplementary Materials and Methods.