Sodium Stibogluconate Interacts with IL-2 in Anti-Renca Tumor Action via a T Cell-Dependent Mechanism in Connection with Induction of Tumor-Infiltrating Macrophages

Keke Fan,* Ming Zhou, ‡ Manas K. Pathak,* Daniel J. Lindner, §§ Cengiz Z. Altuntas, † Vincent K. Tuohy, † Ernest C. Borden, *§ and Taolin Yi2*§

IL-2 therapy results in 10–20% response rates in advanced renal cell carcinoma (RCC) via activating immune cells, in which the protein tyrosine phosphatase Src homology 2 domain-containing phosphatase 1 (SHP-1) is a key negative regulator. Based on finding that sodium stibogluconate (SSG) inhibited SHP-1, the anti-RCC potential and action mechanism of SSG and SSG/IL-2 in combination were investigated in a murine renal cancer model (Renca). Despite its failure to inhibit Renca cell proliferation in cultures, SSG induced 61% growth inhibition of Renca tumors in BALB/c mice coincident with an increase (2-fold) in tumor-infiltrating macrophages (Mφ). A combination of SSG and IL-2 was more effective in inhibiting tumor growth (91%) and inducing tumor-infiltrating Mφ (4-fold), whereas IL-2 alone had little effect. Mφ increases were also detected in the spleens of mice treated with SSG (3-fold) or SSG/IL-2 in combination (6-fold), suggesting a systemic Mφ expansion similar to those in SHP-deficient mice. T cell involvement in the anti-Renca tumor action of the combination was suggested by the observations that the treatment induced spleen IFN-γ T cells in BALB/c mice, but failed to inhibit Renca tumor growth in athymic nude mice and that SSG treatment of T cells in vitro increased production of IFN-γ capable of activating tumoricidal Mφ. The SSG and SSG/IL-2 combination treatments were tolerated in the mice. These results together demonstrate an anti-Renca tumor activity of SSG that was enhanced in combination with IL-2 and functions via a T cell-dependent mechanism with increased IFN-γ production and expansion/activation of Mφ. Our findings suggest that SSG might improve anti-RCC efficacy of IL-2 therapy by enhancing antitumor immunity.

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Renal cell carcinoma (RCC) is a malignant disease with ~31,200 new cases and 12,000 deaths each year in the United States (1). A large portion of RCC patients have initially, or develop after treatment of localized carcinoma, advanced disease (2) that is poorly responsive to conventional treatments, including chemotherapy and radiation therapy (3). These patients have a median survival rate of only 8 mo and a 5-year survival rate of <10% (3). Immunotherapy (4), based on activation of antitumor immunity using cytokines or immune cells, has been investigated as an alternate systemic approach for the treatment of advanced disease (2) that is poorly responsive to conventional treatments, including chemotherapy and radiation therapy (3). These patients have a median survival rate of only 8 mo and a 5-year survival rate of <10% (3). Immunotherapy (4), based on activation of antitumor immunity using cytokines or immune cells, has been investigated as an alternate systemic approach for the treatment of advanced RCC. Among the cytokines evaluated, IL-2 was shown to induce response rates of 10–20% in advanced RCC patients and has been approved for RCC treatment (5, 6).

IL-2 is an activator of T lymphocytes and other types of immune cells (7). It binds to a tripartite receptor complex on cell surfaces to trigger an intracellular signaling cascade that is down-regulated by several mechanisms, including dephosphorylation of IL-2-signaling molecules by protein tyrosine phosphatases (PTPases) (7, 8). Small chemical inhibitors of these negative regulatory PTPases could be expected to enhance the anti-RCC effects of the cytokine and may have potential for immunotherapy. However, this concept of inhibiting negative regulatory PTPases to improve the anti-RCC efficacy of IL-2 therapy has not been directly tested due to the lack of suitable inhibitors against the target PTPases.

Sodium stibogluconate (SSG) was identified recently as a clinically usable PTPase inhibitor that has antimelanoma potential in combination with IFNs (9, 10). SSG is an anti-Leishmania drug with a previously undefined mechanism of action (11). Our recent studies have demonstrated for the first time that SSG inhibits selective PTPases (9), among which the Src homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1) was the most sensitive (9). SSG inactivated recombinant SHP-1 at 10 μg/ml (9), a level comparable to the SSG therapeutic dosage (20 mg/kg) (11). SSG also inhibited PTPases SHP-2 and PTP1B at higher doses, but lacked effects on MKP1 (9). SHP-1 has been established as a key negative regulator of intracellular signaling of cytokines, including IL-2, and immune cell activation (12). Consistent with inhibition of a negative signaling regulator, SSG augmented signaling and cell responses to IL-3, erythropoietin, and IFNs in vitro (9). This action mechanism of SSG in vivo was indicated by its interaction with IFN-α in a synergistic manner to eradicate WM9 melanoma tumors in nude mice (10). Inhibition of a negative regulatory PTPase of intracellular signaling may also play a role in the drug’s anti-Leishmania action. This action mechanism provides a rational explanation for the dependency of SSG anti-Leishmania efficacy on host cytokines and immune cells (13–15) and for the selective drug sensitivity of the intracellular form, but not the free-living

*Department of Cancer Biology, ‡Department of Immunology, Lerner Research Institute, §Taufiss Cancer Center, The Cleveland Clinic Foundation, Cleveland, OH 44195

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2 Address correspondence and reprint requests to Dr. Taolin Yi, Department of Cancer Biology, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, N14-67, Cleveland, OH 44195. E-mail address: yit@ccf.org

3 Abbreviations used in this paper: RCC, renal cell carcinoma; Mφ, macrophage; PTP, protein tyrosine phosphatase; SHP-1, Src homology 2 domain-containing phosphatase 1; SSG, sodium stibogluconate.
type, of the pathogen (16). The relevance of SHP-1 as an SSG target has also been suggested by observations that SHP-1 was required for intracellular survival of the pathogen (17) and that PT-Pase was activated during Leishmania infection to attenuate cytokine signaling (18, 19).

Given the negative regulatory role of SHP-1 in IL-2 signaling and immune cell activation, SSG might induce anti-RCC immunity and interact with IL-2 to improve the anti-RCC efficacy of IL-2 therapy. As an initial step to assess this potential of SSG as a novel anti-RCC agent, we investigated the activity and mechanism of action of SSG, alone and in combination with IL-2, against renal tumors (Renca) in mice. We report for the first time a potent anti-Renca tumor activity of SSG that interacts with IL-2 in a tolerated manner to inhibit Renca tumor growth and functions via a T cell-dependent mechanism coincident with induction of tumor-infiltrating macrophages (Mφ) and systemic Mφ expansion. These results provide the first preclinical proof of concept evidence that SSG could be combined with IL-2 to induce more effective anti-RCC immune action and suggest that refined SHP-1 inhibitors could be developed as safe and efficacious immune activators for therapeutic purposes.

Materials and Methods

Reagents, cells, cell culture, and cell growth inhibition assays

Renca (20), Jurkat (21), and WM9 (22) cell lines were obtained from a colleague at the Cleveland Clinic Foundation and cultured in RPMI 1640 medium supplemented with 10% FCS. Recombinant IL-2 (Proleukin; 22 million IU/1.3 mg; Chiron) was purchased from the Cleveland Clinic Foundation pharmacy. SSG has been described previously (10). For cell growth inhibition assays, cells were cultured in the absence or the presence of various amounts of SSG for 6 days, with viable cells quantified by MTT assays as described previously (9).

Animal studies

BALB/c and athymic nude BALB/c mice (10 wk old, female; Taconic Farms) were inoculated (s.c.) at the flanks with Renca cells (10^6 cells/site). Four days after inoculation, the mice were subjected to no treatment (control) or treatment with IL-2 (10^5 IU/day for 5 days i.p.), SSG (12 mg/day i.m., at hip regions), or the combination of the two agents for 2 wk. The IL-2 dose was comparable to those used in previous studies for assessing murine anti-Renca tumor immunity (23). The dose of SSG was similar to the effective daily dose of the drug for the treatment of murine leishmaniasis (24). As the first study to evaluate the efficacy and toxicity of IL-2/SSG treatment in the absence of comparison with other regimens, the scheme of the individual agents were used in combination based on their proven safety and efficacy in mice. Tumor volume was measured during the study period and calculated using the formula for a prolate spheroid (V = 4/3 * π * a * b) (25). Student’s t test was used for assessing the significance of tumor volume differences among differential treatment groups. Mouse viability (daily) and body weights (weekly) were also recorded during the study period. At the end of the study, Renca tumors and major internal organs (heart, kidney, liver, lung, and spleen) were harvested for histology and immunohistochemistry analysis.

Histology and immunohistochemistry

Major internal organs and Renca tumors harvested from mice were fixed in 10% formalin or snap-frozen in liquid nitrogen. H&E-stained tissue sections of the fixed samples were prepared and evaluated by microscopy as described previously (10). Preparation of frozen tissue sections and immunohistochemistry were performed following established procedures (26). The Abs used for immunohistochemistry were anti-CD4 (rat mAb, clone GK1.5; BD Biosciences), anti-CD8 (rat mAb, clone 53-6.7.5; BD Biosciences), anti-F4/80 (rat mAb, clone A3-1; Serotec), and anti-asialo-GM1 (rabbit polyclonal; Cedarlane Laboratories). Irrelevant mAbs with isotypes matching F4/80 or other specific mAbs were used as negative controls and showed no staining for the samples under the experimental conditions used. The sections were counterstained with Mayer’s hematoxylin before microscopic examination. Tissue sections of two mice per group were evaluated. The number of immune cells was semiquantitated based on the following scheme: ≥, zero to one positive cell per ×40 field; +, two to five positive cells per ×40 field; ++, six to 10 positive cells per ×40 field, etc.

Quantification of IFN-γ cells by ELISPOT assays and of IFN-γ by ELISA

BALB/c mice (10 wk old, female; Taconic Farms) were subjected to no treatment (control) or treatment with the combination of IL-2 (2 × 10^5 IU i.p.) and SSG (12 mg i.m.) for 4 days before they were killed, and spleens were harvested. Single-cell suspensions of splenocytes were prepared and used in ELISPOT assays to quantify IFN-γ cells using a commercial ELISPOT kit (R&D Systems) following the manufacturer’s procedure. The scanning and counting of the IFN-γ spot-forming cells were accomplished with an automatic ELISPOT reader and Immunospot2 software (Cellular Technology). Splenocytes from mice treated with the combination of SGG/IL-2 were also separated into populations expressing or lacking expression of CD4 and CD8 surface markers (CD4+ /CD8+ and CD4- /CD8-) using microbeads conjugated with Abs against mouse CD4 and CD8a (Miltenyi Biotec) as reported previously (27). The cell populations were then used in ELISPOT assays for quantification of IFN-γ cells as described above. To assess the effects of SSG and IL-2 on mouse splenocytes in vitro, splenocytes from untreated BALB/c mice were suspended in RPMI 1640 medium supplemented with 10% FCS and incubated in the absence or the presence of SSG (20 μg/ml), IL-2 (30 U/ml), or their combination for 16 h before ELISPOT assays for quantification of IFN-γ cells. To determine whether SSG affects IFN-γ secretion from T cells, Jurkat cells (5 × 10^5 cells/ml) were suspended in RPMI 1640 medium supplemented with 10% FCS. The cell suspension was cultured in the absence or the presence of various amounts of SGG for 16 h. Culture medium supernatants were harvested after centrifugation (1000 × g, 10 min). The amounts of IFN-γ in the culture medium supernatants were quantified using an ELISA kit (R&D Systems) following the manufacturer’s protocol.

Results

SSG inhibits Renca tumor growth in BALB/c mice, but not Renca cell proliferation in culture

To investigate a potential anti-RCC activity of SSG that functions via an immune mechanism, the effects of SSG on Renca tumor growth in BALB/c mice were determined. Renca, derived from a spontaneous kidney tumor in BALB/c mice, was chosen based on its tumorigenicity in this strain of immune-competent mice (20).

Initially, the effects of SSG on Renca cell growth in culture were examined to determine whether SSG could directly inhibit Renca cell growth in the absence of immune cells. Renca cells cultured in the absence or the presence of SSG (6.25–200 μg/ml) for 6 days showed similar growth (Fig. 1A), whereas the growth of WM9 melanoma cells was inhibited by SSG in a dose-dependent manner under comparable conditions (Fig. 1B) as reported previously (10).

The effects of SSG on Renca tumor growth in vivo were assessed by treating BALB/c mice bearing 4-day-established Renca tumors with SSG, which was administered daily for a 2-wk period at its effective dosage for murine leishmaniasis (24). At the end of the treatment period, Renca tumors in the SSG-treated mice were significantly (p < 0.01) smaller (39%) than those (100%) in the untreated controls (Fig. 2). The treatment was tolerated without evident toxicity in the mice, which all survived at the end of treatment (data not shown).

FIGURE 1. Differential growth responses of Renca and WM9 cells to SSG in vitro. Renca (A) and WM9 (B) cells were cultured in the absence or the presence of various amounts of SSG for 6 days. Viable cells were then quantified by MTT assays. Data represent the mean ± SD of triplicate samples.
SSG/IL-2 in combination induces more effective Renca tumor growth inhibition than single agents

A putative antitumor immune mechanism for SSG in anti-Renca tumor action suggested that Renca tumor growth might be inhibited more effectively by SSG in combination with IL-2, which is known to activate antitumor immune cells (7). Because the cytokine is an approved treatment and induces low response rates in advanced RCC (28), evidence that SSG interacts with IL-2 in Renca tumor growth inhibition would also provide preclinical proof of concept regarding the potential of SSG to improve the efficacy of IL-2 therapy. The effects of SSG/IL-2 in combination on Renca tumor growth were therefore determined.

BALB/c mice bearing 4-day-established Renca tumors were treated with an SSG/IL-2 combination or with IL-2 alone and compared with untreated controls and mice subjected to SSG treatment. The SSG/IL-2 combination treatment for 2 wk induced 90% of Renca tumor growth inhibition vs 61% induced by SSG ($p < 0.01$; Fig. 2). However, the combination did not eradicate tumors under the experimental conditions, because all the treated mice had small residual tumors, which resumed growth in mice observed for a longer period (data not shown). IL-2 as a single agent under the experimental conditions failed to inhibit Renca tumor growth (Fig. 2), consistent with previous reports (23, 29). The combination was tolerated, as indicated by the survival of all the treated mice at the end of the treatment period, the comparable body weight gains of untreated and treated mice, as well as the absence of histopathological changes in major organs of the treated mice at the time of death (data not shown).

These results suggested that SSG/IL-2 in combination induced more effective Renca tumor growth inhibition than the agents individually, consistent with an immune mechanism of SSG anti-Renca tumor action. The capacity of SSG to interact with IL-2 in anti-Renca tumor action and the tolerance of the combination treatment in mice suggest the possibility of SSG/IL-2 combination therapy as an improved treatment for advanced RCC.

SSG and SSG/IL-2 treatments induce tumor-infiltrating MΦ and systemic MΦ expansion

To define the mechanism of action of SSG and SSG/IL-2 in combination on Renca tumor growth inhibition, the effects of these treatments on Renca tumor-infiltrating immune cells were investigated. T, NK, and MΦ lineage cells are important antitumor effectors (4). The relative numbers of these immune cells in Renca tumors from mice differentially treated with SSG, IL-2, or their combination were quantified by immunohistochemistry.

T lymphocytes (CD4+ or CD8+) were present in low numbers in Renca tumors, as reported previously, and showed little difference among tumors from the differentially treated mice (Fig. 3A), whereas NK cells were undetectable in the tumors (data not shown) under the experimental conditions used. Although tumor-infiltrating MΦ (F4/80+) were at comparable levels in control and IL-2-treated mice, interestingly, a modest increase (∼2-fold) occurred in SSG-treated mice, and a more marked increase (∼4-fold) was seen in SSG/IL-2-treated mice (Fig. 3).

To assess whether the increased MΦ in Renca tumors from SSG- and SSG/IL-2-treated mice was a tumor-specific event or part of a systemic MΦ expansion, the relative numbers of MΦ in spleens of the differentially treated mice were also quantified by immunohistochemistry. Spleen MΦ (F4/80+) numbers were significantly increased (∼3-fold) in SSG-treated mice and markedly increased (∼6-fold) in mice treated with the SSG/IL-2 combination compared with background levels of MΦ in control or IL-2-treatment groups (Fig. 4). Spleens of the differentially treated mice showed similar levels of CD4+/CD8+ cells (Fig. 4A).

These results demonstrated that SSG treatment induced MΦ infiltration in Renca tumors and systemic MΦ expansion, amplified by coadministration of IL-2. In contrast, SSG or SSG/IL-2 treatment had little effect on the numbers of tumor-infiltrating T cells or NK cells. This selective induction of tumor-infiltrating MΦ and systemic MΦ expansion by SSG and SSG/IL-2 provides histological evidence supporting an immune mechanism for the anti-Renca tumor action of the treatments and implicates MΦ as potential direct anti-Renca tumor effector cells.
identified an increase in M\textsubscript{tumors} and spleens from the control and SSG/IL-2-treated mice in athymic mice. Immunohistochemical analysis of Renca of growth inhibitory activity of SSG/IL-2 treatment on Renca tumors were untreated or treated with SSG/IL-2 in combination for 2 wk. Renca tumors grew in a comparable manner in both groups from the differentially treated BALB/c mice (Fig. 2) as quantified by immunohistochemistry. Tissue sections of spleens harvested from mice at the end of the treatments were stained with anti-CD4, anti-CD8, or anti-F4/80 mAb. The CD4\textsuperscript{+}, CD8\textsuperscript{+}, and F4/80\textsuperscript{+} cells in the spleens from treated mice were scored (fold increase) by comparison with the basal levels in the spleens of control mice. 

**Discussion**

The results presented in this study demonstrate for the first time a significant anti-Renca tumor activity of SSG that is mediated via an immune mechanism and augmented in the presence of IL-2.

An immune mechanism of SSG anti-Renca tumor action was supported by several lines of evidence. It was suggested by the initial observation that SSG induced Renca tumor growth inhibition in BALB/c mice, but failed to inhibit Renca cell proliferation in culture, which argues against a direct anti-Renca tumor mechanism via SSG cytotoxicity. It was also indicated by the histological evidence that SSG induced tumor-infiltrating M\textsubscript{φ} expansion. It was supported strongly by the genetic evidence of T cell requirement for the anti-Renca tumor action of SSG/IL-2 in combination and by the capacity of this combination to induce IFN-\textgamma\ T cells in vivo. We showed in previous studies an antimelanoma tumor activity of SSG that probably resulted from a direct action of SSG on tumor cells, because SSG inhibited the growth of melanoma cells in culture and inhibited melanoma tumor growth in immune-deficient nude mice (10). The capacity of SSG to interact with recombinant human IFN-\alpha in antimelanoma tumor action in mouse models probably resulted from an immunity-independent mechanism based on the synergy of SSG/IFN-\alpha in direct growth inhibition of melanoma cells in culture in the absence of immune cells (10). Involvement of IFN-activated immunity in the reported antimelanoma tumor action in mice could be excluded, because of the species specificity of recombinant human IFN-\alpha, which is inactive on mouse immune 

**FIGURE 4.** SSG and SSG/IL-2 combination treatments increase spleen M\textsubscript{φ} in BALB/c mice. A. Relative numbers of T cells and M\textsubscript{φ} in spleen from the differentially treated BALB/c mice (Fig. 2) as quantified by immunohistochemistry. Tissue sections of spleens harvested from mice at the end of the treatments were stained with anti-CD4, anti-CD8, or anti-F4/80 mAb. The CD4\textsuperscript{+}, CD8\textsuperscript{+}, and F4/80\textsuperscript{+} cells in the spleens from treated mice were scored (fold increase) by comparison with the basal levels in the spleens of control mice. B. Representative views (\times 20) of F4/80\textsuperscript{+} cells in spleens from the differentially treated mice.

**SSG/IL-2 anti-Renca tumor action requires the presence of T cells**

A putative mechanism that M\textsubscript{φ} act as direct anti-Renca tumor cells does not exclude an involvement of T cells, which might be activated by SSG or SSG/IL-2 to secrete cytokines required for inducing the tumoricidal activity of M\textsubscript{φ}. To assess the role of T cells in the anti-Renca tumor action of the combination, the anti-Renca tumor efficacy of SSG/IL-2 was studied in athymic mice lacking T cells.

Athymic nude BALB/c mice bearing 4-day-established Renca tumors were untreated or treated with SSG/IL-2 in combination for 2 wk. Renca tumors grew in a comparable manner in both groups of mice during the treatment period (Fig. 5), demonstrating a lack of growth inhibitory activity of SSG/IL-2 treatment on Renca tumors in athymic mice. Immunohistochemical analysis of Renca tumors and spleens from the control and SSG/IL-2-treated mice identified an increase in M\textsubscript{φ} in tumors (2-fold) and spleen (3-fold) from SSG/IL-2-treated mice compared with control values (data not shown).

To assess the role of T cells in SSG/IL-2 action, the effects of the combination on the number of IFN-\gamma T cells in BALB/c mice were determined. SSG/IL-2 treatment for 4 days induced a significant in-

**FIGURE 5.** Effects of IL-2/SSG combination treatment on Renca tumor growth in athymic BALB/c mice. Renca cells were inoculated (10\textsuperscript{6} cells/site s.c.) into athymic BALB/c mice (nu/nu). The mice with 4-day-established Renca tumors were then untreated (controls) or treated with the combination of IL-2 (10\textsuperscript{3} IU/day i.p.) and SSG (12 mg/day i.m.). Renca tumor volumes (mean ± SD; \(n = 5\)) in these mice were recorded as indicated. The treatment durations are indicated by the arrows.

**FIGURE 6.** Induction of spleen IFN-\gamma cells by the SSG/IL-2 combination in BALB/c mice and in vitro. A, Splenocytes of BALB/c mice untreated (controls) or treated with SSG/IL-2 in combination (IL-2/SSG) for 4 days were harvested and suspended in RPMI 1640 culture medium. IFN-\gamma cells in splenocytes were quantified by ELISPOT assays. RPMI 1640 culture medium was used as a background control. B, Splenocytes of BALB/c mice treated with SSG/IL-2 for 4 days were separated, using microbeads, into populations lacking the expression of CD4 and CD8 surface markers (CD4\textsuperscript{+} or CD8\textsuperscript{+}) or expressing either or both these molecules (CD4\textsuperscript{+} or CD8\textsuperscript{+}). IFN-\gamma cells in the populations were quantified by ELISPOT assays. RPMI 1640 culture medium was used in ELISPOT assays as a background control. C, Splenocytes from untreated BALB/c mice were incubated in the absence (untreated) or the presence of SSG, IL-2, or their combination for 16 h before quantification of IFN-\gamma cells by ELISPOT assays. Data represent the mean ± SD of duplicate samples.
cells, but would inhibit the growth of melanoma cells of human origin (10). Our current study thus revealed a novel immune-mediated antitumor action of SSG.

The finding of an immune-mediated antitumor action of SSG has significant clinical implications in addition to providing insights into the mechanism of action of the drug. It suggests a broader SSG application as a potential anticancer therapeutic that might be beneficial in patients with tumors sensitive as well as insensitive to direct growth inhibition by SSG. Moreover, the dual antitumor actions of SSG via direct tumor growth inhibition and antitumor immunity suggest that SSG might be most effective when used in immune-competent patients with tumors sensitive to direct SSG growth inhibition. This concept might aid in the selection of cancer patients for optimal efficacy of SSG-based therapy and could be verified in future preclinical studies. In addition, the immune-mediated antitumor action of SSG suggests the potential of SSG to be used in combination with other immune activation agents, including IL-2, which was investigated in this study.

The SSG/IL-2 combination was demonstrated in this study to be more effective in anti-Renca tumor action than the single agents. The superior anti-Renca tumor action of the SSG/IL-2 combination and the tolerance of the treatment in mice provided preclinical proof-of-concept evidence that SSG might have potential for improving the efficacy of IL-2 anti-RCC therapy and warrants its clinical evaluation in the future. In this regard, it is worth noticing that the dose of IL-2 used in our anti-Renca tumor experiment was ~25% of the reported maximal tolerated dose in mice (29). Tolerance of SSG with IL-2 at the maximal tolerated dose in mice was also observed in a preliminary experiment (our unpublished observations). It is therefore possible that more striking anti-Renca tumor action might be achievable using optimized SSG/IL-2 combination therapy that could be defined through differential dosing and/or treatment schedules. The demonstrated capacity of SSG to interact with IL-2 without obviously increasing IL-2 toxicity, which is mediated through IL-2-activated T cells that induce a capillary leaking syndrome (7), might be related to our observation that SSG anti-Renca tumor activity is probably mediated in part through Mφ, as discussed below, and thus does not depend solely on increasing IL-2-induced T cell activation.

An important finding of our study was that SSG induced tumor-infiltrating Mφ and a marked systemic Mφ expansion, which were amplified by IL-2. In addition to providing histological evidence supporting an immune mechanism for SSG anti-Renca tumor action, this SSG activity was a potential indication of in vivo inhibition of SHP-1 in SSG-treated mice, because systemic Mφ expansion is a key feature of mice with genetic SHP-1 deficiency (32–34). Moreover, it implicates Mφ as direct antitumor effector cells of the drug. Such a putative role for Mφ was consistent with the apparent lack of effect of SSG on the levels of tumor-infiltrating T cells and was supported by previous reports of Mφ as important immune cells with tumoricidal activity (30, 35). Significantly, it also provides a rational explanation for the SSG/IL-2 interaction in anti-Renca tumor action. Because the induction of tumor-infiltrating Mφ by SSG was augmented by IL-2, the heightened anti-Renca tumor effects of SSG/IL-2 in combination might have resulted from a converging action of the two agents on Mφ that directly attacks the tumor cells. However, it is not clear at present how IL-2 augmented SSG-induced tumor-infiltrating Mφ. Although IL-2R is expressed on monocytes (36) that differentiate into Mφ, our observation that the effect of IL-2 on tumor-infiltrating Mφ was T cell dependent argues against a direct role for IL-2-induced monocyte differentiation. The involvement of cytokines from IL-2-activated T cells in the process is a more likely alternative mechanism. Future studies using mice with deficiencies in selective cytokines or immune cell subpopulations will help to define the mechanism of SSG/IL-2-induced Mφ expansion and its role in anti-Renca tumor action.

In addition to revealing a putative role for Mφ in SSG anti-Renca tumor action, our results implicated the involvement of T cells that are known to play a key role in antitumor immunity (4). T cells were apparently required for the capacity of IL-2 to augment SSG induction of tumor-infiltrating Mφ and systemic Mφ expansion. This was indicated by the observation that the levels of tumor-infiltrating Mφ and spleen Mφ expansion in SSG/IL-2-treated athymic mice were similar to those induced by SSG alone in the T cell-competent BALB/c mice. The importance of T cells is further underscored by the lack of Renca tumor growth inhibition in the presence of the modest increase in tumor-infiltrating Mφ in SSG/IL-2-treated athymic mice. Taking into consideration the low number of tumor-infiltrating T cells and the capacity of SSG to induce T cell secretion of IFN-γ capable of activating Mφ (30, 31), the results together suggest that T cells might mediate SSG/IL-2 anti-Renca tumor action through secreting cytokines to induce and activate tumor-infiltrating Mφ.

Our finding that SSG exerts anti-Renca tumor activity via an immune mechanism is also significant in several other aspects. It provides evidence that strengthens a postulated immune mechanism of SSG in anti-Leishmania action. In particular, the observed systemic Mφ expansion in SSG-treated mice suggests the presence of such a pharmacological effect during SSG antileishmaniasis therapy that might have been overlooked previously. Given the differential activities of SSG against the free-living promastigotes and intracellular amastigotes (16), this raises the possibility that several other compounds (11) with similar anti-Leishmania characteristics might also have potential anticancer activity through immune action and need to be re-evaluated accordingly. Taking into consideration the tolerance of SSG and its apparent capacity to activate immune cells by inhibiting SHP-1, it is possible that refined inhibitors of the phosphatase could be developed as safe immune activators for anticancer therapy and other immune therapies.

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Disclosures
T. Yi has filed patent applications on sodium stibogluconate.

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