Induction of Interleukin 3 and Tumor Resistance by SSM, a Cancer Immunotherapeutic Agent Extracted from Mycobacterium tuberculosis

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ABSTRACT

Interleukin 3 (IL-3) activity was demonstrated when inguinal lymph node cells obtained from Bacillus Calmette-Guérin-sensitized mice (BCG-ILNC) were stimulated in vitro with SSM, an immunomodulator extracted from Mycobacterium tuberculosis. The IL-3 activity was first detected on Day 1 in culture fluids of BCG-ILNC stimulated with SSM, reached a peak on Day 3, and then gradually decreased. The activity was completely neutralized by treatment with anti-murine IL-3 monoclonal antibody (mAb). When BCG-ILNC were treated with anti-Thy 1.2 or anti-Lyt 1.2 mAb followed by complement, IL-3 was not produced in the culture fluids. However, IL-3 in the culture fluids was detected when BCG-ILNC were treated with anti-Lyt 2.2 mAb, anti-asialo-GM1, or anti-mouse immunoglobulin antiserum followed by complement. These results suggested that Lyt 1* T-cells appeared to be required for the production of IL-3 from BCG-ILNC stimulated with SSM. In addition, low but significant IL-3 activity was also observed in sera of mice treated with SSM. However, serum IL-3 activity was not detected in mice treated with both SSM and Thy 1.2 or Lyt 1.2 mAb, whereas the activity was induced by SSM in mice treated with anti-Lyt 2.2 mAb or anti-asialo-GM1 antiserum. On the other hand, the in vivo growth of IMC tumors inoculated in BALB/c x DBA/2 F1 mice was significantly decreased by intrasplenic injection of culture fluids containing IL-3, as well as by SSM itself. This antitumor activity of the culture fluids was not altered when it was treated with mAbs for interleukin 1, interleukin 2, or anti-mouse γ-interferon antiserum. The antitumor activity of the fluid was only eliminated when it was treated with anti-mouse IL-3 mAb. Since nonspecific resistance to tumors in mice stimulated with SSM appears to require Lyt 1* T-cells, these results suggest that, in part, nonspecific resistance to tumors of mice stimulated with SSM may be developed through IL-3, which was produced by Lyt 1* T-cells after SSM stimulation.

INTRODUCTION

SSM, an arabinomannan preparation extracted from Mycobacterium tuberculosis strain Aoyama B (1), has been shown to have immunotherapeutic activity in patients with malignancy (2). The antitumor activity of SSM has also been demonstrated in rats bearing ascites hepatoma (3), in BALB/c mice bearing RL61 leukemia (4) and Ehrlich carcinoma (4), and in BALB/c × DBA/2 F1 mice (hereafter called CD2F1) mice bearing IMC RLX1 leukemia (4) and Ehrlich carcinoma (5). Although it is known that the mechanism of antitumor action, no chemotherapeutic antitumor activities of SSM were demonstrated (6). Suzuki et al. (4) reported that a NSRT of mice stimulated with the agent was not observed in mice depleted with Lyt 1* T-cells. This result suggests that NSRT stimulated with SSM may be expressed through the function of these T-cells. Although, among the T-cell populations, cytotoxic T-lymphocytes have been described as antitumor effector cells, the antitumor activity of helper T-cells has also been reported. Specific cytotoxic activities that were mediated by Lyt 1* T-cells (mouse) or OKT 4* T-cells (human) were generated by in vitro long-term cultures (7, 8). Helper T-cells are also described as being involved in the tumor rejection caused by adoptive immunotherapy (9, 10). These cells may express their efector functions independently of that of cytotoxic T-lymphocyte generation.

By the way, SSM has been shown to stimulate the production of soluble factors such as IL-1 (11), colony-stimulating factor (12), and IFN-γ (13). A mitogenic factor that stimulates the proliferation of SSM-sensitized lymphocytes has been also demonstrated in cultures of helper T-cells treated with SSM (14). Since many papers described (15) the generation of various anticancer effector cells in several conditions by stimulation with lymphokines or cytokines, it may be possible that these soluble factors released from SSM-treated cells play a role on the expression of NSRT of mice stimulated with SSM. Although SSM could stimulate production of IFN-γ (13), it has been demonstrated (16) that NSRT-stimulating activity of SSM was not related to the induction of IFN-γ in tumor-bearing hosts. Therefore, in the present study, other soluble factors that could be involved in the development of the NSRT in tumor-bearing mice stimulated with SSM were investigated. IL-3 activity in culture fluids of SSM-stimulated inguinal lymph node cells from BCG sensitized mice was detected, and its importance in the NSRT of mice stimulated with SSM was studied.

MATERIALS AND METHODS

Mice, Cells, and Media. Six-week-old CD2F1 (Charles River, Wilmington, MA) and 6- to 8-week-old C57BL/6 (Jackson Laboratories, Bar Harbor, ME) mice were used in the experiments. FDC-P2 cells, an IL-3-dependent cell line (17), were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Hazelton Research Products, Lenexa, KS), γ-glutamyl (3 mmol), penicillin (100 units/ml), streptomycin (100 µg/ml), and 10% conditioning medium obtained from cultures of myelomonocytic leukemia WEHI-3 cells, an IL-3-producing cell line (18). The culture fluids containing IL-3 were prepared by incubating 1 × 10⁶ cells/ml of WEHI-3 cells in RPMI 1640 medium supplemented with 10% fetal bovine serum, γ-glutamyl, and antibiotics (growth medium) for 48 h at 37°C in 5% CO2 (18). The IMC carcinoma, established at the Institute of Microbial Chemistry in Japan (19) and kindly provided by Nomura Research Institute of Life Science, Kanagawa, Japan, was serially passed in CD2F1 mice by i.p. injection of 1 × 10⁶ cells/mouse.

Reagents. SSM (Lot 8A13), supplied by Zeria Pharmaceutical Co., Ltd., Tokyo, Japan, was dissolved in growth medium at various concentrations. In antitumor experiments in mice, SSM dissolved in 0.01 M PBS at concentrations ranging from 0.1 to 1 mg/ml was injected intrasplenically into solid tumor-bearing mice at an amount of 0.2 ml/mouse. To deplete Thy 1* T-cells, Lyt 1* T-cells, Lyt 2* T-cells, cells expressing asialo GM1, antigen (mainly natural killer cells), or B-cells from inguinal lymph node cells, we treated BCG-ILNC, as described previously (4, 20), with anti-Thy 1.2 mAb (ICN Biochemicals, Costa Mesa, CA), anti-Lyt 1.2 mAb (Accurate Chemical Scientific Co.,...
with SSM, respectively. Anti-IFN-γ was provided from the Department of Microbiology, The University of Texas Medical Branch at Galveston. Anti-IL-3 mAb (rat monoclonal anti-mouse IL-3-neutralizing antibody; rat IgG2b 19B3.1) was a gift from Dr. J. S. Abrams, the DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA. The specificity of neutralizing activities of anti-IFN-γ and anti-IL-3 mAb has been shown previously (21, 22).

Induction and Assay of IL-3. For the production of IL-3, the single cell suspension of BCG-ILNC was prepared from BCG-sensitized C57BL/6 mice according to the report of Alkan et al. (23). In these experiments, ILNC were prepared from mice 10 days after infection with 125 μg/mouse of BCG. The ILNC were purified by Ficoll-Hypaque gradient centrifugation (density, 1.074 g/ml) (24). The ILNC of C57BL/6 mice not sensitized with BCG (normal-ILNC) were prepared by the same procedures. BCG-ILNC and normal-ILNC were suspended in growth media at a concentration of 5 × 10^6 cells/ml and cultured at 37°C for 1 to 4 days in 5% CO_2 in the presence or absence of a 100-μg/ml dose of SSM. Culture fluids harvested on various days after incubation were used as a putative source of IL-3. In some experiments, the BCG-ILNC were washed 3 times with growth medium after 3-h incubation to remove SSM and cultured for 3 more days in fresh growth medium. Then it was harvested. Samples were kept at −70°C until assays were performed. The production of IL-3 in mice treated with SSM was also examined in vivo. In accordance with previous descriptions (12), serum specimens were harvested from CD2F1 mice 6 h after the administration of SSM (10 mg/kg, i.p.). The IL-3 activity was assayed by using a microradiation technique with a murine IL-3-dependent cell line, FDC-P2 cells (25). The FDC-P2 cells were extensively washed with growth medium and suspended in the same medium at a concentration of 1 × 10^6 cells/ml. The mixture of this cell suspension (1 × 10^6 cells/well) and undiluted assay samples was seeded at a volume of 100 μl each in 96-well microtiter plates (Corning, No. 25860) and cultured at 37°C for 24 h in 5% CO_2. IL-3 activity was detected by the growth of FDC-P2 cells as determined by the [3H]thymidine incorporation technique (25), and the results were expressed the average of five wells per sample.

Depletion of Immunocompetent Cells. To deplete Thy 1+ T-cells, Lyt 1+ T-cells, Lyt 2+ T-cells, or cells expressing asialo-Gm, antigen in vivo, CD2F1 mice were treated with anti-Thy 1.2 mAb (2.5 × 10^6 units/kg, i.p.), anti-Lyt 1.2 mAb (1.7 × 10^6 units/kg, i.p.), anti-Lyt 2.2 mAb (1 × 10^6 units/kg, i.p.) or anti-asialo-Gm, antiserum (5 mg/kg, i.v.) without exogenous complement, respectively (26-28). One unit of mAb activity is equivalent to the antibody titer required for 20% cytotoxicity in vitro.

RESULTS

Induction of IL-3 by SSM in Vitro. Cultures fluids of BCG-ILNC or normal-ILNC (5 × 10^6 cells/ml) stimulated with a 100-μg/ml dose of SSM were assayed for their IL-3 activities. As shown in Fig. 1, culture fluid harvested 3 days after incubation with SSM showed significant levels of IL-3 activity, but the activity was not observed in the fluids of BCG-ILNC not stimulated with SSM. Although normal-ILNC stimulated with SSM also produced IL-3 activity in their culture fluids (P < 0.05), the activity was much lower than that of BCG-ILNC. In addition, the IL-3 activity in culture fluid of BCG-ILNC cultured for 3 days, in which SSM was washed out after 3-h exposure, was also demonstrated (Fig. 1). The kinetics of IL-3 production from ILNC stimulated with SSM is shown in Fig. 2. The IL-3 activity was first detected on Day 1 in culture fluids of BCG-ILNC stimulated with SSM, reached a peak on Day 3, and then gradually decreased.

To determine whether activity found in the culture fluids was IL-3, we performed a neutralization test using anti-IL-3 mAb. Anti-IL-3 mAb (15 ml at 1:100 dilution) was mixed with an equal volume of the culture fluid containing IL-3 activity. The mixture was kept at 37°C for 1 h, and then FDC-P2 cells were

% of inhibition = \left(1 - \frac{\text{cpm of treated group}}{\text{cpm of control group}}\right) × 100

% of reduction of IL-3 production

Antitumor Experiments. CD2F1 mice were inoculated i.d. with IMC tumor cells (1 × 10^6 cells/mouse) into the shaved left side of the abdomen. Three days after the tumor inoculation, 0.2 ml of 0.01 M PBS containing appropriate doses of SSM were administered directly into the tumor sites. The treatment of tumor-bearing mice was continued twice a week for a total of six injections. To determine antitumor activity of the culture fluids containing IL-3 activity, which were obtained from cultures of BCG-ILNC 3 days after incubation with SSM, tumor-bearing mice were treated with the culture fluid using the same schedule as SSM itself. Tumor sizes were measured twice a week using a microlipper to determine tumor diameters. Tumor sizes were expressed as the product of the largest diameter (a) and the smallest diameter (b) of the solid tumors, i.e., a × b mm^2. The antitumor activities of tested samples were evaluated on the basis of the inhibition percentage of tumor growth calculated utilizing the following formula.

% of reduction of IL-3 production

Neutralization of IFN-γ, IL-1, IL-2, or IL-3 Activity. Fifteen ml of culture fluids containing IL-3 activity were mixed with an equal volume of anti-IFN-γ (400 IFN-γ-neutralizing units/ml) (21), a 1:2 dilution of anti-IL-1 mAb, a 1:2 dilution of anti-IL-2 mAb (30), a 1:100 dilution of anti-IL-3 mAb (22), or medium, respectively, and mixtures were kept for 1 h at 4°C. The antitumor activity of these culture fluids was determined in mice bearing IMC solid tumors, as described above.

Statistical Analysis. Student's t test was used to analyze the difference between treated groups and controls. A P value less than 0.05 was considered significant.

RESULTS

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% of inhibition = \left(1 - \frac{\text{mean tumor size of treated group}}{\text{mean tumor size of control group}}\right) × 100

% of inhibition

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INTERLEUKIN 3 PRODUCED BY SSM STIMULATION

Table 1 Effect of anti-IL-3 mAb treatment on the IL-3 activity in culture fluids of BCG-ILNC stimulated with SSM

<table>
<thead>
<tr>
<th>Culture fluids were treated with</th>
<th>IL-3 activity (cpm)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>14,042 ± 2,860°</td>
<td>87°</td>
</tr>
<tr>
<td>Anti-IL-3 mAb</td>
<td>1,823 ± 610°</td>
<td></td>
</tr>
</tbody>
</table>

*Culture fluids of BCG-ILNC incubated with SSM (100 µg/ml) for 3 days at 37°C were treated with anti-IL-3 mAb (1:100 dilution) as described in the text.

**IL-3 activity was determined by the [3H]thymidine uptake method using FDC-P2 cells.

For treatment of the culture fluids with anti-IL-3 mAb, a reduction of IL-3 activity was calculated using the following formula

Reduction (%) = \( \frac{cpm \text{ of mAb-treated group} - cpm \text{ of control group}}{cpm \text{ of control group}} \times 100 \).

* Mean ± SD.

** P < 0.001 (Student's t test).

The Nature of IL-3 Producer Cells. To determine which cells were responsible for the IL-3 production, BCG-ILNC were treated with various antibodies plus complement and, then, stimulated with SSM as described above. Results are shown in Fig. 3. Treatment of BCG-ILNC with anti-Thy 1.2 mAb followed by complement appeared to decrease IL-3 activity by 96% (P < 0.001), whereas elimination of Lyt 1+ T-cells from BCG-ILNC by treatment with anti-Lyt 1.2 mAb and complement resulted in a 75% decrease of the IL-3 production (P < 0.001). However, treatment of BCG-ILNC with anti-asialo-GM, antiserum, anti-Lyt 2.2 mAb, or antiimmunoglobulin antiserum followed by complement did not affect the IL-3 production of cells stimulated with SSM. These results strongly suggest that IL-3 was produced from Lyt 1+ T-cells stimulated with SSM, whereas Lyt 2+ T-cells, B-cells, and cells expressing asialo-GM, appeared not to be involved in the IL-3 production stimulated by SSM.

IL-3 Activities in Sera of Mice Treated with SSM. To determine whether IL-3 was produced in mice by SSM administration, we obtained serum specimens from CD2F, mice 6 h after injection of SSM at a dose of 10 mg/kg i.p. As shown in Table 2, there was a slightly significant increase in the level of IL-3 activity in serum of mice given injections of SSM, as compared with that of the SSM-untreated control (P < 0.05). To determine which cells were responding to the IL-3 production in vivo, mice were treated with anti-Thy 1.2, anti-Lyt 1.2, and anti-Lyt 2.2 mAbs or anti-asialo-GM, antiserum as described above. As shown in Table 2, after stimulation with SSM, the amount of IL-3 in the circulation of mice treated with anti-Thy 1.2 or anti-Lyt 1.2 mAb was decreased as compared with that of appropriate controls. However, the IL-3 production stimulated by SSM was unchanged in mice treated with anti-Lyt 2.2 mAb or anti-asialo-GM, antiserum as compared with that of control.

NSRT of Mice Stimulated with SSM or SSM-induced IL-3. Three days after tumor inoculation (IMC solid tumor), 10

![Graph showing IL-3 activity in culture fluids of BCG-ILNC](image1)

![Graph showing IL-3 activity in sera of mice treated with SSM](image2)
CD2F, mice were each treated intralesionally with a 1- and 10-
mg/kg dose of SSM. As a control, 20 CD2F, mice were treated
with PBS (0.2 ml/mouse) as described in “Materials and Meth-
ods.” Twenty-one days after tumor inoculation, tumor sizes
were measured using the microcaliper. The results showed that
85% (10 mg/kg) or 39% (1 mg/kg) of tumor growth was
inhibited by SSM administration as compared with that of the
control mice (Fig. 4). Although, in this experiment, SSM was
injected directly into the tumor site, the observed suppression
of tumor growth was thought to be due to the NSRT of mice
stimulated with SSM, because SSM itself has no cytotoxicity
against tumor cells directly (6). To determine the NSRT-stim-
ulating activity of SSM-induced IL-3, CD2F, mice bearing IMC
solid tumors were treated intralesionally with 0.2 ml/mouse
of the culture fluid containing IL-3 activity, as described above.

As shown in the results in Fig. 5, the growth of solid tumors
was clearly reduced by the treatment of the culture fluid as
compared with the control. The inhibition rate was calculated
as 57% at 24 days after tumor inoculation (P < 0.001). Similar
antitumor activity of the culture fluid of BCG-ILNC, which
were exposed to SSM for a 3-h period of the 3-day incubation,
was also demonstrated in mice bearing the same IMC solid
tumors (data not shown).

To determine whether NSRT-stimulating activity of the cul-
ture fluids was mediated by IL-3 itself, we intralesionally
injected CD2F, mice bearing solid tumors with: (a) PBS (0.2 ml/
mouse, a tumor control); (b) SSM (1 mg/kg, a general positive
control); (c) culture fluids (0.2 ml/mouse, a positive control);
(d) culture fluids treated with anti-IL-1 mAb, anti-IL-2 mAb,
anti-IL-3 mAb, or anti-IFN-γ antiserum, respectively. As a
negative control, tumor-bearing mice were treated with various
mAbs or antiserum alone in the schedules as described.

As shown in Table 3, NSRT-stimulating activity of the culture
fluids was clearly abolished by the treatment with anti-IL-3
mAb, but not by other mAbs or antiserum. At controls, SSM
and the culture fluid themselves stimulated NSRT of mice
bearing IMC tumors, and antibodies themselves also did not
show any affect on the tumor growth in the same mice.

DISCUSSION

SSM is a potential immunotherapeutic anticancer agent (2–
5) with various biological activities, which include activation of
the reticuloendothelial system (31) and protection against X-
ray-induced leukopenia (32). The precise antitumor mecha-
nisms of SSM have been undetermined. However, because SSM
has not been shown to have a direct cytotoxic effect against
tumor cells in vitro (6) and the antitumor effect of SSM has
been transferred to SSM-untreated mice by spleen cells (5), this
antitumor effect could be expressed through the development
of NSRT of tumor-bearing hosts stimulated with SSM. On the
other hand, SSM stimulated the production of various cyto-

Fig. 4. Antitumor activity of SSM in mice bearing IMC solid tumors. CD2F,
mice were inoculated i.d. with IMC tumor cells (1 × 10⁶ cells/mouse). Three days
after tumor implantation, mice were treated intralesionally with SSM at a dose
of 1 mg/kg (C, 10 mice) or 10 mg/kg (△, 10 mice). The treatment was continued
twice a week for a total of six injections. As a control, tumor-bearing mice were
treated intralesionally with 0.2 ml/mouse of PBS (○, 20 mice) by the same
schedules with SSM. Points, mean; bars, SD.

Fig. 5. Effect of culture fluids of BCG-ILNC which contain IL-3 on the growth
of IMC solid tumors in CD2F, mice. CD2F, mice were inoculated i.d. with IMC
tumor cells (1 × 10⁶ cells/mouse). Three days after tumor inoculation, these mice
were treated intralesionally with 0.2 ml/mouse of the culture fluid of BCG-ILNC
(C, 10 mice) or the same amount of PBS (○, 20 mice). The treatment schedules
and measurement technique of tumor sizes were described in the text. Points,
mean, bars, SD.
interleukin 3 produced by SSM stimulation

Table 3 Effects of mAb treatments on the antitumor activity of culture fluids of BCG-ILNC which contain IL-3 activity

<table>
<thead>
<tr>
<th>Tumor-bearing mice were treated intrasplenically witha</th>
<th>No. of mice</th>
<th>Mean tumor sizeb (mm²)</th>
<th>Inhibition of tumor growth (%)c</th>
<th>Pd</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (tumor control)</td>
<td>30</td>
<td>109.4 ± 54.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSM (general control)</td>
<td>10</td>
<td>57.0 ± 39.8</td>
<td>48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Culture fluids (positive control)f</td>
<td>10</td>
<td>45.6 ± 28.5</td>
<td>58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Culture fluids +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-IL-1 mAb</td>
<td>10</td>
<td>43.4 ± 20.6</td>
<td>60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-IL-2 mAb</td>
<td>10</td>
<td>48.2 ± 23.1</td>
<td>56</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Anti-IL-3 mAb</td>
<td>10</td>
<td>103.9 ± 35.4</td>
<td>5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Anti-IFN-γ antiserum</td>
<td>10</td>
<td>46.2 ± 26.2</td>
<td>58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Medium +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-IL-1 mAb</td>
<td>10</td>
<td>122.5 ± 48.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anti-IL-2 mAb</td>
<td>10</td>
<td>121.4 ± 38.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anti-IL-3 mAb</td>
<td>10</td>
<td>113.8 ± 48.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anti-IFN-γ antiserum</td>
<td>10</td>
<td>111.5 ± 63.6</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

a IMC tumor-bearing CD2F mice were treated intrasplenically with PBS (0.2 ml/mouse), SSM (1 mg/kg), culture fluids (0.2 ml/mouse), or a 1:1 mixture (0.4 ml/mouse) of the culture fluids and antibodies, or antibody alone (0.2 ml/mouse) as described in the text.
b Twenty-four days after tumor inoculation, tumor sizes were measured by a microcaliper, and they were expressed by the mean of mm² obtained from length (mm) x width (mm) of the solid tumors.
c Percentage of inhibition of tumor growth calculated using the formula described in the text.
d Student’s t test.
e Mean ± SD.
f Culture fluids of BCG-ILNC incubated with SSM (100 µg/ml) for 3 days were injected into tumor-bearing mice.

Kines or lymphokines (11–14). Among the antitumor mechanisms of certain immunopotentiators against experimental tumors, an important role of cytokines or lymphokines, which appeared in tissues or serum after the administration of immunopotentiators, on the suppression of tumor growth has been reported (15). Lentinan, a β-1,3-glucan with some β-1,6-glucoside side chains extracted from Japanese edible mushrooms, Lentinus edodes, is an antitumor immunotherapeutic agent (33). The antitumor activity of lentinans is expressed through the induction of cytokines such as IL-1 and colony-stimulating factor, associated with inflammatory responses (34). Carboxyethylgermanium sesquioxide (Ge-132), an organogermanium compound with immunomodulating activities, has been shown to exert an antitumor effect in tumor-bearing hosts (35). Because the antitumor effect of Ge-132 could be completely abolished when tumor-bearing mice were treated with anti-IL-3 mAb, a possible mechanism of antitumor action was suggested in which Ge-132 inhibited tumor growth through functions of IFN-γ stimulated with the compound itself (36). Although IFN-γ production is stimulated by SSM treatment (13), IFN-γ was not shown to participate in the antitumor mechanisms of SSM (16), which leads to the possibility that other cytokines or lymphokines may be involved in the antitumor activity of SSM.

In the present study, IL-3 activity released from BCG-ILNC was demonstrated after treatment with SSM in vitro. The activity was identified as IL-3 by a neutralization test using anti-IL-3 mAb. In addition, the growth of syngeneic solid tumors inoculated in mice was significantly suppressed when SSM itself or the culture fluid containing IL-3 activity was administered directly into tumor sites. In addition, this antitumor activity of the culture fluid was eliminated by treatment with anti-IL-3 mAb in vitro. Further, the IL-3 production from BCG-ILNC stimulated with SSM was abolished when Lyt 1+ T-cells were depleted from BCG-ILNC. Since the NSRT-stimulating activity of SSM is expressed through the function of Lyt 1+ T-cells (4), this suggests that the IL-3 induced from Lyt 1+ T-cells by SSM may be important in the development of the NSRT in mice stimulated with SSM.

IL-3 is a member of the family of colony-stimulating factors that regulates hemopoiesis (37). IL-3 has been also reported to stimulate the proliferation and differentiation of hematopoietic progenitor cells (38, 39). Although the exact mechanism of NSRT-stimulating activity of IL-3 has not been determined, it has already been reported that IL-3 could promote the differentiation and expansion of natural cytotoxic killer cells (40, 41). In the present experiments, the culture fluids containing IL-3 activity were administered into tumor sites directly, some effective interactions between cells infiltrated into tumor nodules and IL-3 administered might be considered. Therefore, it is possible that, in part, NSRT of mice stimulated with SSM may be displayed through cells infiltrated into tumor sites; the antitumor activity of tumor-infiltrated lymphocytes was promoted by IL-3 released from Lyt 1+ T-cells by SSM stimulation. Further study will be required to examine a role of IL-3 released from Lyt 1+ T-cells by SSM stimulation on the antitumor functions of effector cells infiltrated into tumor sites.

Acknowledgments

We thank Zeria Pharmaceutical Co., Ltd., for their generous donations of SSM and Dr. J. S. Abrams, the DNAX Research Institute of Molecular and Cellular Biology, for his gift of anti-IL-3 mAb.

References

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