Antitumor Effect of a Splenic Injection of 5-Fluorouracil on Metastatic Liver Cancer in Mice

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ABSTRACT

The regional administration of 5-fluorouracil (5-FU) has been the fundamental therapy against liver metastases for the improvement of patient prognosis; however, there have been few reports about the immunological effects of this agent. It is also unknown whether it affects the spleen, one of the major lymphoid organs. The objective of the present study was to determine the immunological effect of an intrasplenic injection of 5-FU against liver metastases. We investigated the effect of an intrasplenic injection of 5-FU on the formation of experimental liver metastasis resulting from an intraportal vein injection of colon 26 carcinoma cells in BALB/c mice and elucidated some of the underlying mechanisms involving the effects of this on cellular immunity. Liver metastases were significantly diminished by the splenic injection of 5-FU, particularly in comparison with the portal injection or systemic administration. This was followed by augmentation of the interleukin-12 (IL-12) level in the spleen and activation of hepatic mononuclear cells. In those cells, NK1.1+ (NKT) cells played a central role against metastases. A splenic injection of 5-FU is more effective on the involution of liver metastases than portal or systemic injection. This effect may be attributed to the augmentation of the IL-12 level in the spleen and of NKT cells in the liver rather than to the original effect of 5-FU, which is the so-called inhibition of DNA synthesis.

Liver metastasis, the most common recurrence of colon carcinomas, is assumed to be the best indicator of a patient’s prognosis. Currently, although regional administration (hepatic arterial infusion) of anticancer agents is used to prevent postoperative recurrence (also in cases that have unresectable tumors), the results are not always satisfactory. In general, 5-fluorouracil (5-FU)-based chemotherapy is widely considered to be associated with the suppression of the immune function (Mitchell and DeConti, 1970; Berenbaum, 1979); however, other reports indicate that the natural killer (NK) cytotoxicity in the spleen is reinforced by 5-FU (Blomgren et al., 1965; Goto et al., 1981; Okamoto et al., 1998), which is one of the most frequently used anticancer drugs. Therefore, there have been only a few reports about the immunological effect of 5-FU on cancer.

5-FU is a potent inducer of several cytokines, such as interferon-γ, tumor necrosis factor-α and -β, and interleukin-12 (IL-12) (Okamoto et al., 1998). Foremost, IL-12 has been described as a growth factor for activated NK cells and NK1.1+ (NKT) cells (Kobayashi et al., 1989; Perussia et al., 1992; Brunda et al., 1993; Trinchieri, 1994; Hashimoto et al., 1995; Smyth et al., 2000), and these cells have been known to be cytotoxic effector cells and to comprise the main antimetastatic lymphocyte population in the liver (Watanabe et al., 1995; Kobayashi et al., 2002). Meanwhile, systemic administration of the IL-12 protein was reported to augment the number of hepatic mononuclear cells (hMNCs) in mice, suppress the growth of a variety of established tumors in mice, and prolong the survival of tumor-bearing mice (Brunda et al., 1993; Hashimoto et al., 1995; Zou et al., 1995).

The objective of this study is to determine the immunological effect and mechanisms of the intrasplenic effect of 5-FU against liver metastases in mice and to establish whether the administration routes of 5-FU have some relationship with its effects. We investigated the cytotoxicity of splenic cells or hMNCs and the level of IL-12 in the spleen or liver. A subset of hMNCs having an anti-tumor effect was also identified using monoclonal antibodies.

Materials and Methods

This study was approved by the Animal Research Ethics Board of Gunma University, Graduate School of Medicine.

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Materials and Methods

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Mice. Normal inbred BALB/c mice aged 7 weeks were routinely used (Charles River Japan, Tokyo, Japan). All mice were housed in the Institute of Experimental Animal Research, Gunma University, Graduate School of Medicine, under conditions with a laminar air flow. They were maintained on standard laboratory feed and in 12-h light/dark cycles.

Preparation and Administration of Colon Tumor Cells. The mice were anesthetized using diethyl ether and laparotomized using a median incision on day 0. One hundred thousand colon 26 cells, a mouse colon carcinoma cell line capable of liver metastasis of BALB/c mice origin, were obtained by the in vivo selection method (Fidler, 1973) and injected into the portal vein. The wound was closed with continuous sutures of 3-0 silk in layers. Liver metastasis of colon tumor cells was demonstrated on the surface of the liver macroscopically 2 weeks after inoculation.

Administration Routes of 5-FU. The subjects were divided into four groups. On day 14, all mice were anesthetized and laparotomy was performed again. In the first group, 25 mg/kg 5-FU (a kind gift from Kyowa Hakko Co., Tokyo, Japan) was injected directly into the spleen. The second group took the agent injected via the portal vein, the third group, via the common iliac vein. Normal saline was used (Charles River Japan, Tokyo, Japan). All mice were housed in a temperature-regulated environment of Na\textsubscript{2}CO\textsubscript{3} (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK) in PBS, was added to this extract. The enzyme extract of Na\textsubscript{2}CO\textsubscript{3} density gradient was used. Cells at the top band were collected and washed in a cold medium. These cells were considered to be hMNCs, and their total number was recorded. The spleens were also finely minced and placed in a 50-ml polypropylene tube in a cold medium. This extract was placed through a nylon mesh (50 μm; Tokyo Screen Co., Tokyo, Japan). For liver lymphocyte isolation, Percoll (Amersham Biosciences Inc., Piscataway, NJ) density gradient was used. Cells at the top band were collected and washed in a cold medium. These cells were considered to be hMNCs, and their total number was recorded. The spleens were also finely minced and placed in a 50-ml polypropylene tube in a cold medium. This extract was placed through a nylon mesh, and the total number of collected splenic cells was counted.

Antitumor cellular immunity was determined in terms of the NK and CTL cytotoxicity using 5\textsuperscript{1}Cr-release assay. One million colon 26 cells incubated with 100 μg/ml Mitomycin-C (Kyowa Hakko Co., Tokyo, Japan) before use and isolated 1 × 10\textsuperscript{6} hMNCs or splenic cells were mix-cultured at 37°C for 6 days as described previously (Mosley et al., 1989; Rodolfo et al., 1996). For delivered CTL isolation, Ficoll-Paque (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK) was used as the medium. After centrifugation, the lymphocyte layer was collected because it contains the effector cells for the CTL cytotoxicity assay. In this study, the NK-sensitive Molo- ney virus-induced YAC-1 lymphoma cell line of A/Sn mice was used as the target for NK cytotoxicity, and colon 26, as the target for CTL cytotoxicity. These cells were labeled with 100 μCi (per 1 × 10\textsuperscript{6} cells) of Na\textsubscript{2}CrO\textsubscript{4} (1 mCi = 37 MBq/ml; Amersham Biosciences Inc.) for 30 min at 37°C in a complete medium and washed with the medium. Effector cells were splenic cells or hMNCs from each group. The 5\textsuperscript{1}Cr-release assay was performed in 96-well round-bottom tissue culture plates. A graded number of effector cells in 100 μl were mixed with 5 × 10\textsuperscript{5} target cells in 100 μl, with a target-to-effector (T:E) ratio of 1:2.5, 1:5, and 1:10. For the determination of maximum release, five wells of target cells in 100 μl of the medium were mixed with 100 μl of Triton-X (Calbiochem-Novabiochem, San Diego, CA). After 4 h of incubation, the supernatant was harvested and counted using a gamma counter. Cytotoxicity was calculated as the percentage of releasable counts after subtraction of the spontaneous release.

Enzyme-Linked Immunosorbert Assay for IL-12 Using Liver and Spleen Homogenates. Liver and spleen homogenates from 10 mice from each group were collected to measure the IL-12 level using the method of Borovkova et al. (2000) with some modifications. In brief, the spleen was rapidly excised, rinsed of blood, and homogenized by Polytron (Brinkmann Instruments, Westbury, NY) instrumentally in an amount of a homogenization buffer (PBS containing 0.5% Triton-X and a protease inhibitor; pH 7.2; 4°C) that was equal to the liver or spleen weight. Homogenates were centrifuged at 12,000g for 10 min, and supernatants were collected.

To measure the concentration of IL-12 in the serum and homogenates, the enzyme-linked immunosorbert assay kit (BioSource International, Camarillo, CA) was used. The optical density was examined at 450 nm by a microplate reader. The concentration of IL-12 had been calibrated from a dose-response curve based on reference standards. We multiplied the values obtained for samples from the homogenate by 2 to correct for the 1:2 dilutions.

In Vivo Cell Depletion. To investigate the principal cells that have high NK cytotoxicity, starting 2 days before the tumor inoculation, other tumor-implanted mice received an intraperitoneal injection of 50 μg/mouse anti-asialoGM1 (ASGM1) Ab (Wako Pure Chemicals, Tokyo, Japan) or 200 μg/mouse anti-NK1.1 Ab (BD Pharmingen, San Diego, CA) every 6 days (Seki et al., 1997; Kawamura et al., 1999). Each antibody was administered by 60 mice. Twenty mice from each group were injected with 5-FU on day 14 by one of three methods: intrasplenic, intraportal, or intravenous injection. These mice were sacrificed on day 18. The liver weight and the number of metastatic foci on the surface of each fixed liver was counted. The very small number of metastatic foci were counted by microscopic analysis.

Cytotoxic T Lymphocyte (CTL) and NK Cytotoxicity Assay of hMNCs and Splenic Cells. Hepatic MNCs were isolated using the method of Wiltrout et al. (1984), with some modifications. Four days after injection of 5-FU (on day 18), 10 mice from each group were sacrificed, and the liver and the spleen were extracted. The liver was minced into small pieces and centrifuged, and the supernatant was removed. A prewarmed enzyme solution, containing 0.005% collagenase type 4 (Sigma-Aldrich, St. Louis, MO) and 500 η/ml DNase type 1 (Biozyme Laboratories, Ltd., Gwent, South Wales, UK) in PBS, was added to this extract. The enzyme extract mixture was incubated at 37°C for 30 min, and the enzymatic digest was washed in a cold medium and centrifuged. The cell suspension was passed through a nylon mesh (50 μm; Tokyo Screen Co., Tokyo, Japan). For liver lymphocyte isolation, Percoll (Amersham Biosciences Inc., Piscataway, NJ) density gradient was used. Cells at the top band were collected and washed in a cold medium. These cells were considered to be hMNCs, and their total number was recorded. The spleens were also finely minced and placed in a 50-ml polypropylene tube in a cold medium. This extract was placed through a nylon mesh, and the total number of collected splenic cells was counted.

Statistical Analyses. To determine the significance of the difference between the experimental groups concerning total cell number (TCN), organ weight, the number of metastatic foci, and cytotoxicity, analysis of variance and post hoc test (Bonferroni/Dunn test) were used. Survival rates were calculated by the method of Kaplan-Meier. p values of <0.05 were judged to be statistically significant.

Results

Effect of 5-FU Injection into the Spleen Influences Spleen and Liver Weight after Inoculation of Tumor Cells. Spleen and liver weight were examined after the inoculation of colon 26 to investigate the effect of 5-FU injection into the spleen on liver metastases. As shown in Table 1, in three groups that were injected with 5-FU, the average weight of the spleen in the group with an intrasplenic injec-
In the same way, the number of metastatic foci on the surface of the liver
significantly in comparison with the other groups (p < 0.001). Furthermore, a significantly greater extension of the survival period was observed in the three groups that received 5-FU compared with that in the group receiving normal saline (p < 0.001) (Fig. 1). However, the number of days that the animals survived did not differ significantly among the three groups that received 5-FU.

To investigate the condition of cell proliferation in the spleen and the liver, the number of splenic cells and hMNCs was counted. The total number of splenic cells in the group with an intrasplenic injection of 5-FU was significantly more abundant in the other groups that received 5-FU (p < 0.001) (Table 2). But there was not a significant difference between the group with an intrasplenic injection of 5-FU and normal saline. Although the number of cells per spleen weight was significantly smaller in the group that received intravenous injections of 5-FU compared with the other groups (p < 0.001), that in the group injected with 5-FU into the spleen was not significantly larger than that in the group with an intraportal injection of 5-FU or with an intrasplenic injection of normal saline. With respect to the total number of hMNCs, there was no significant difference among all groups. To express the relative increase or the capacity of hMNCs that attack metastatic foci, the number of hMNCs per liver weight was calculated. The number in the group injected with 5-FU into the spleen was significantly higher than that in the other groups (p < 0.01) (Table 3). These results suggest that the intrasplenic injection of 5-FU with a single 25-mg/kg dose plays a critical role in the involution of liver metastases and prolongs the survival period through the augmentation of hMNCs in comparison with the other groups. They also suggest that the injection to the spleen restrains the reduction of splenic cells as the systemic administration of 5-FU.

**Cytotoxic Activities of hMNCs and Splenic Cells.** We examined the cytotoxicity of splenic cells and hMNCs isolated from mice in all groups using Yac-1 lymphoma cells and colon 26 as targets. There was no significant difference in the CTL and NK cytotoxicity of splenic cells among the groups (data not shown). The same result was obtained with regard to the CTL cytotoxicity of hMNCs in each group (data not shown). On the other hand, the NK cytotoxicity of hMNCs in the group injected with 5-FU into the spleen was significantly increased in comparison with that in the other groups in any point of the T:E ratio (p < 0.001) (Fig. 2). In particular, the difference was nearly twice as high as that in the group with an intrasplenic injection of normal saline. These results suggest that the injection of 5-FU into the spleen augments the NK cytotoxicity of hMNCs but not that of splenic cells. Furthermore, they demonstrate that the intrasplenic injection of 5-FU does not augment the CTL cytotoxicity of both spleen and liver.

**Up-Regulation of IL-12 by Treatment with 5-FU into the Spleen.** Because IL-12 reportedly acts as a growth factor for activated NK cells, we examined the IL-12 level in spleen and liver homogenates of mice after 5-FU administration. As shown in Fig. 3, the IL-12 level in spleen or liver in the group

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**TABLE 1**

<table>
<thead>
<tr>
<th>Spleen Weight</th>
<th>Liver Weight</th>
<th>No. of Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrasplenic (5-FU)</td>
<td>183.7 ± 13.7a</td>
<td>2527.9 ± 359.2b</td>
</tr>
<tr>
<td>Intraportal (5-FU)</td>
<td>144.8 ± 24.6</td>
<td>2980.0 ± 281.7</td>
</tr>
<tr>
<td>Intravenous (5-FU)</td>
<td>119.1 ± 14.7</td>
<td>3616.0 ± 654.8</td>
</tr>
<tr>
<td>Intrasplenic (saline)</td>
<td>217.6 ± 21.7</td>
<td>3503.5 ± 675.2</td>
</tr>
</tbody>
</table>

*p < 0.001 versus the other groups.

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**TABLE 2**

<table>
<thead>
<tr>
<th>TCN of Splenic Cells</th>
<th>TCN/Sp spleen weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(× 10⁶)</td>
</tr>
<tr>
<td>Intrasplenic (5-FU)</td>
<td>82.3 ± 6.5a</td>
</tr>
<tr>
<td>Intraportal (5-FU)</td>
<td>61.7 ± 7.1</td>
</tr>
<tr>
<td>Intravenous (5-FU)</td>
<td>43.4 ± 8.0a</td>
</tr>
<tr>
<td>Intrasplenic (saline)</td>
<td>92.3 ± 15.3</td>
</tr>
</tbody>
</table>

*a p < 0.001 versus intraportal (5-FU) and intravenous (5-FU).

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**TABLE 3**

<table>
<thead>
<tr>
<th>TCN of hMNCs</th>
<th>TCN/Liver Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(× 10⁶)</td>
</tr>
<tr>
<td>Intrasplenic (5-FU)</td>
<td>2.2 ± 0.3a</td>
</tr>
<tr>
<td>Intraportal (5-FU)</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Intravenous (5-FU)</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Intrasplenic (saline)</td>
<td>2.1 ± 0.4</td>
</tr>
</tbody>
</table>

*a Not significant versus the other groups.

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**Fig. 1.** Effects of intrasplenic injection of 5-FU on survival in the metastatic model. The heavy line represents the cytotoxicity in the group under intraportal injection of 5-FU, the normal line represents that under intravenous injection of 5-FU, and the dotted line represents that under intrasplenic injection of normal saline. A significantly greater extension of the survival period was observed in groups that received 5-FU into the spleen compared with normal saline (p < 0.001). However, there were no significant differences among the three groups that received 5-FU. Intraportal (5-FU) versus intrasplenic (saline), p < 0.001. Intravenous (5-FU) versus intrasplenic (saline), p < 0.01.
injected 5-FU into the spleen was significantly higher than the levels in the other groups (p < 0.001). Particularly, the IL-12 level in the liver was nearly twice as high in the group with intrasplenic injection with 5-FU as it was in the other groups. These facts support the findings that the splenic injection of 5-FU stimulates the splenic immune system to produce IL-12 and up-regulate the IL-12 level in the liver.

**Involvement of NK Cells and NKT Cells.** To investigate the principal cells that have high NK cytotoxicity, we used two antibodies, which depleted NK cells or both NK and NKT cells. Anti-ASGM1 Ab depletes only NK cells, and anti-NK1.1 Ab depletes NK and NKT cells. The liver weight and number of metastatic foci of liver in the group pretreated with anti-ASGM1 Ab and injected with 5-FU into the spleen were significantly less than those in the other groups (p < 0.01) (Table 4). The NK cytotoxicity of hMNCs in the group injected with 5-FU into the spleen was significantly higher than that in other groups in any point of the T:E ratio (p < 0.001). Moreover, the NK cytotoxicity of hMNCs (Fig. 5) in the group injected with rmIL-12 via the portal vein were higher than those in the groups that received an intravenous injection of rmIL-12 and an intrasplenic injection of normal saline (p < 0.05). These facts show that, in spite of the augmentation of the NK cytotoxicity by injection of IL-12 through the portal vein, the liver weight and the number of metastases did not diminish as they did when IL-12 was administered into the spleen.

**Discussion**

The liver is one of the most susceptible organs to metastasis of a digestive cancer, especially colon cancer. Currently, various treatments, such as resection, microwave coagulation, and local administration of anticancer agents, are used. Although it is obvious that resection is the most effective method to prevent metastasis and recurrence in the liver, the local administration of anticancer agents, such as 5-FU, is still potentially important for long-term prognosis.

The spleen is a large lymphoid organ that produces various kinds of cytokines (Hood et al., 1978), which are known to stream into the liver via the splenic and portal veins and to enhance NK cytotoxicity in the liver (Shiratori et al., 1995). NK cells have been known to mediate spontaneous cytotoxicity against tumor cells and their metastases (Gorelik et al., 1982). It has also been reported that hepatic NK cells act as the first line of defense in the hepatic metastasis of colon cancer (Shiratori et al., 1992; Bouwens et al., 1998).

The splenic cells from mice given 5-FU intraperitoneally...
to be associated with the suppression of immune function (at
reduces the number and the volume of metastases.
possibility that an intrasplenic injection of 5-FU can prolong
portal or intravenous injection of 5-FU. These facts reveal the
saline, and at least, no less than that in mice with an intra-
significantly longer than that in mice injected with normal
spleen, the weight of the liver, and the number of metastases
were diminished. Furthermore, hMNCs increased, and their
immune system in the spleen is up-regulated and whether it
exhibited higher NK activities (Okamoto et al., 1998). The
present study was carried out to determine whether the
immune system in the spleen is up-regulated and whether it
has an anticancer effect when 5-FU is administered into the
spleen. As a result, in the mice that received 5-FU into the
portal vein was significantly higher (\( p < 0.001 \)) than those in the other groups. Moreover, the liver level in the group injected with rmIL-12 into
the portal vein was significantly higher (\( +++\), \( p < 0.001 \)) than that in the group with an intravenous injection of rmIL-12 and intrasplenic injection of
normal saline. Error bars represent S.D.

### TABLE 4
Number of metastatic foci on the surface of liver

<table>
<thead>
<tr>
<th>Liver Weight (mg)</th>
<th>No. of Metastases</th>
<th>NK Cytotoxicity of hMNCs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T:E = 1:2.5</td>
<td>T:E = 1:5</td>
</tr>
<tr>
<td>ASGM1 + intrasplenic (5-FU)</td>
<td>2751.3 ± 339.1(^{a})</td>
<td>23.7 ± 7.6(^{b})</td>
</tr>
<tr>
<td>ASGM1 + intraportal (5-FU)</td>
<td>3557.4 ± 423.5</td>
<td>36.0 ± 8.2</td>
</tr>
<tr>
<td>ASGM1 + intravenous (5-FU)</td>
<td>3311.7 ± 361.7</td>
<td>37.3 ± 8.5</td>
</tr>
<tr>
<td>NK1.1 + intrasplenic (5-FU)</td>
<td>3622.8 ± 541.8</td>
<td>36.4 ± 7.3</td>
</tr>
<tr>
<td>NK1.1 + intraportal (5-FU)</td>
<td>3730.4 ± 688.3</td>
<td>39.2 ± 6.7</td>
</tr>
<tr>
<td>NK1.1 + intravenous (5-FU)</td>
<td>3426.3 ± 484.0</td>
<td>37.9 ± 9.5</td>
</tr>
</tbody>
</table>

\( ^{a} p < 0.001 \) versus ASGM1 + intraportal (5-FU). 
\( ^{b} p < 0.001 \) versus the other groups.

### TABLE 5
Effects of rmIL-12 on liver metastases

<table>
<thead>
<tr>
<th>Liver Weight (mg)</th>
<th>No. of Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrasplenic (rmIL-12)</td>
<td>2541.5 ± 289.3(^{a})</td>
</tr>
<tr>
<td>Intraportal (rmIL-12)</td>
<td>3235.8 ± 351.6</td>
</tr>
<tr>
<td>Intravenous (rmIL-12)</td>
<td>3485.5 ± 348.1</td>
</tr>
<tr>
<td>Intraspinal (saline)</td>
<td>3503.5 ± 678.2</td>
</tr>
</tbody>
</table>

\( ^{a} p < 0.001 \) versus the other groups.

\( ^{b} p < 0.01 \) versus the other groups.

**Fig. 4.** Distribution of IL-12 after injection of rmIL-12 was examined using enzyme-linked immunosorbent assay. A, IL-12 level obtained from the splenic homogenate. B, IL-12 level obtained from the hepatic homogenate. The spleen and liver levels of IL-12 in the group injected with rmIL-12 into the spleen were significantly higher (\( +++\), \( p < 0.001 \)) than those in the other groups. Moreover, the liver level in the group injected with rmIL-12 into the portal vein was significantly higher (\( +++\), \( p < 0.001 \)) than that in the group with an intravenous injection of rmIL-12 and intrasplenic injection of normal saline. Error bars represent S.D.

### Fig. 5.
Effects of rmIL-12 for liver metastases. Groups shown are defined as follows: \( \mathbf{1} \), intrasplenic injection of rmIL-12; \( \mathbf{l} \), intraportal injection of rmIL-12; \( \mathbf{A} \), intravenous injection of rmIL-12; and \( \times \), intrasplenic injection of normal saline. The NK cytotoxicity of hMNCs in the group injected with rmIL-12 into the spleen was significantly higher than that in the other groups (\( +++\), \( p < 0.001; ***, p < 0.01 \)). Moreover, that in the group injected with rmIL-12 into the portal vein was significantly higher than that in the group with an intrasplenic injection of rmIL-12 and intraspinal injection of normal saline (\( +++\), \( p < 0.001; +, p < 0.05 \)). Error bars represent S.D.

least, transiently) (Mitchell and DeConti, 1970; Berenbaum,
1979), other reports have described the increase in the im-
mune function to result from the inhibition of suppressor T
cell cytotoxicity associated with this drug, inversely
(Blohmgren et al., 1965; Goto et al., 1981, Okamoto et al.,
1998). Thus, the effect of 5-FU on the immune system is
controversial. In this study, the total number of cells per
spleen weight was significantly diminished only in mice that
were injected with 5-FU via the common iliac vein. And that in mice injected with 5-FU into the spleen is not diminished in comparison with the control. These results suggest that splenic cells are involved in the effect on the involution of liver metastases.

Until a certain size, a metastatic tumor in the liver grows by securing nutrients through the hepatic artery (Izumi et al., 1986). Archer and Gray (1990) reported that branches of the portal vein at the tumor periphery become invaded and compressed during the growth of metastases, which further diminishes drug delivery to the tumor site. In addition, 5-FU is an agent with a short half-life in serum of several tens of minutes. Therefore, it is conceivable that there is a new effect other than the original anticancer effect with this method. These findings suggest that the splenic administration of 5-FU reduces the metastatic lesion by augmenting the NK cytotoxicity of hMNCs rather than by the original effect of 5-FU, which is the so-called inhibition of DNA synthesis.

5-FU is a potent inducer of several T helper 1-type cytokines, such as interferon-γ, tumor necrosis factor-α and -β, and IL-12, and of effector cells carrying anticancer cytotoxicity mediated by NK cells as well as T cells; in addition, these abilities are closely associated with the in vivo anticancer effect of this agent (Okamoto et al., 1998). Especially, IL-12 was originally described as a NK stimulatory factor (Kobayashi et al., 1989) or a cytotoxic lymphocyte maturation factor (Trinchieri, 1994) produced principally by antigen-presenting cells. In this study, a 5-FU injection into the spleen was found to augment the level of IL-12 in the spleen or liver more than in the other groups. Furthermore, the fact that the increase of IL-12 is not followed by the augmentation of splenic cytokotoxicity substantiates the view that most of the IL-12 produced in the spleen flows into the liver via the spleen vein and induces an increase in the number of hMNCs and augmentation of the cytokotoxicity of hMNCs. Eberl and MacDonald (2000) reported that the proportion of NK and NKT cells made up about 8% of the total splenic cells and about 35% of the hMNCs. Their findings tend to substantiate this view. Actually, the cytokotoxicity of hMNCs and the IL-12 level in the liver of mice that received injections of 5-FU into the spleen were significantly higher than those in mice that received 5-FU via the other administration routes.

Recent studies on IL-12 show that it acts as a growth factor for activated NK cells and NKT cells (Perussia et al., 1992; Smyth et al., 2000). Watanabe et al. (1995) reported that NKT cells, which proliferate in the liver after the administration of IL-12, are cytotoxic effector cells and comprise the main ant metastatic lymphocyte population in the liver. The systemic administration of IL-12 markedly activates hepatic NKT cells and induces the cytokotoxic activities of hMNC against targets (Brunda et al., 1993; Hashimoto et al., 1995; Smyth et al., 2000). Wiltrout et al. (1984) reported that metastatic lesions became enlarged in mice that were treated by anti-ASGM1 Ab. Seki and colleagues reported that in vivo anti-ASGM1 Ab treatment only depletes NK cells in mice, whereas anti-NK1.1 Ab treatment depletes NK and NKT cells (Seki et al., 1997; Kawamura et al., 1999). This study revealed that antitumor effects persist and NKT cells play a major role in preventing metastases, even when NK cells are depleted and only NKT cells exist.

Systemic administration of IL-12 protein significantly suppressed the growth of a variety of established mouse tumors and prolonged the survival of tumor-implanted mice (Brunda et al., 1993; Zou et al., 1995). The number of hMNCs in mice that received rmIL-12 increased more significantly than that in mice that did not receive it (Hashimoto et al., 1995). In this study, to determine whether IL-12 influences the activation of hepatic NKT cells, rmIL-12 was injected into the spleen in tumor-implanted mice. The results revealed that the rmIL-12 injected into the spleen activated the hepatic NKT cells and reduced the volume of metastases. However, in spite of the augmentation of the NK cytotoxicity by rmIL-12 flowing through the portal vein, the effect did not directly aid in tumor involution. These data imply that some other factor, which is produced in the spleen by IL-12, plays a supplementary role.

We believe that the use of the spleen as a potential reservoir for the activation of cell-mediated immunity in vivo should be taken into account when considering the development of clinical anticancer immunotherapeutic strategies.

References


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