Prolonged Decrease of Serum Calcium Concentration by Murine \( \gamma \)-Interferon in Hypercalcemic, Human Tumor (EC-GI)-bearing Nude Mice

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ABSTRACT

Murine \( \gamma \)-interferon (MuIFN-\( \gamma \)) is a potent inhibitor of bone resorption induced by interleukin 1 and parathyroid hormone-related protein in vitro. To investigate whether MuIFN-\( \gamma \) is also effective in vivo, the cytokine was injected s.c. into hypercalcemic tumor (EC-GI)-bearing nude mice, in which parathyroid hormone-related protein and interleukin 1a are synergistically responsible for causing humoral hypercalcemia.

When MuIFN-\( \gamma \) was injected s.c. at a dose of 1 to 20 \( \times 10^4 \) units for 5 days consecutively, serum calcium concentrations in the tumor-bearing mice decreased in a dose-dependent manner. The minimal effective dose was 5 \( \times 10^4 \) units/mouse. Unlike calcitonin, which decreased the serum calcium concentration for only 1 to 2 days despite continuous daily injections, MuIFN-\( \gamma \) decreased it for more than 7 days even after the injections had been stopped. Human \( \gamma \)-interferon was completely ineffective. The decrease in serum calcium concentration was accompanied by a decrease in urinary calcium excretion. Histological examination of the femur revealed a decreased number of osteoclasts in the MuIFN-\( \gamma \)-treated mice. Furthermore, MuIFN-\( \gamma \) when injected into nude mice or normal mice at a dose of 15 \( \times 10^4 \) units for 3 days, almost completely abolished the formation of multinucleated osteoclast-like cells in vitro.

These findings suggest that MuIFN-\( \gamma \) suppresses the formation and maturation of osteoclasts and inhibits osteoclastic bone resorption, resulting in the prolonged decrease of serum calcium concentration seen in hypercalcemic, tumor-bearing nude mice. Therefore, bone resorption inhibitors like MuIFN-\( \gamma \), which ameliorate humoral hypercalcemia without an escape phenomenon, are potentially useful for the treatment of malignancy-associated hypercalcemia.

INTRODUCTION

Malignancy-associated hypercalcemia is a most frequent paraneoplastic syndrome. Although calcitonin and glucocorticoids can usually decrease the serum calcium concentration of hypercalcemic patients with malignant disorders, the effect is only transient (1). Therefore, more potent agents to control hypercalcemia for a prolonged period are to be developed.

MuIFN-\( \gamma \) is a potent inhibitor of bone resorption in vitro (2, 3). Recently we demonstrated that MuIFN-\( \gamma \) produces ubiquitous and prolonged inhibition of bone resorption induced by either IL-1 or PTHrP in fetal mouse forearm bones (4). Unlike calcitonin, which inhibits bone resorption for only a few days, MuIFN-\( \gamma \) completely inhibits \( ^{44} \)Ca release induced by PTHrP or IL-1\( \alpha \) without any escape phenomenon (4). Therefore, IFN-\( \gamma \), a pluripotent cytokine (5, 6) with properties including enhancement of the expression of major histocompatibility complex type II antigens and activation of macrophages, natural killer cells, and antitumor activity, may also be useful for the treatment of malignancy-associated hypercalcemia.

Recently we established a tumor cell line from a patient with humoral hypercalcemia (EC-GI) and showed that it produced PTHrP and IL-1\( \alpha \) (7, 8). Nude mice bearing the tumor invariably developed humoral hypercalcemia, in which both PTHrP and IL-1\( \alpha \), acting synergistically, increase the concentration of serum calcium (9).

To investigate whether MuIFN-\( \gamma \) is also effective in vivo, we administered it to hypercalcemic tumor (EC-GI)-bearing nude mice and compared its therapeutic effect with that of calcitonin for treatment of humoral hypercalcemia of malignancy.

MATERIALS AND METHODS

Animals, Tumor Cells, and Drugs. Five-wk-old nude mice (BALB/c or ICR strain) were purchased from Animal Laboratories (Shizuoka, Japan). EC-GI-10 cells producing PTHrP and IL-1\( \alpha \) (1 to 2 \( \times 10^7 \) cells) were inoculated into the right flank of each mouse. As the tumor grew, reaching a size of about 1 cm\(^3\), the serum calcium concentration gradually increased. The tumor had been successively transplanted into 6-wk-old BALB/c nude mice for the last 3 yr. In some experiments, the tumor was transplanted into ICR nude mice. Recombinant MuIFN-\( \gamma \) (specific activity, 3 \( \times 10^6 \) units/mg of protein; Lot 2271-54-F3) (10) and recombinant HuIFN-\( \gamma \) were kindly supplied by Daiichi Pharmaceutical Co. (Tokyo, Japan) and Shionogi Pharmaceutical Co. (Osaka, Japan), respectively. The cytokines were stored at 4°C in concentrated form and diluted immediately prior to use. Salmon calcitonin (Samotin, 10 units/vial) and eel calcitonin (Elctetonin, 10 units/vial) were supplied by Yamanouchi Pharmaceutical Co. (Tokyo, Japan) and Toyo Jozo Pharmaceutical Co. (Tokyo, Japan), respectively. Human PTHrP(1-34) was purchased from the Peptide Institute (Osaka, Japan).

Effect of Calcitonin on Serum Calcium Concentration in Hypercalcemic Nude Mice. Salmon and eel calcitonin were diluted with 0.9% NaCl solution and injected s.c. into tumor-bearing, hypercalcemic nude mice at a daily dose of 0.1 to 1 unit/0.2 ml of saline once a day for 11 to 17 days. Control tumor-bearing nude mice received only vehicle (0.2 ml of 0.9% NaCl solution).

One, 3, 5, 8, and 11 days after injection, the mice were lightly anesthetized with ether, and blood was obtained from the orbita. About 200 \( \mu \)l of blood from each mouse were taken into heparinized capillary tubes (Drummond Scientific Co., Broomall, PA), and the serum calcium concentration was determined using a colorimetric assay kit (RM 117-K; Iatron Laboratories, Inc., Tokyo, Japan).

Effect of IFN-\( \gamma \) on Serum Calcium Concentration in Hypercalcemic Nude Mice. Each five nude mice (BALB/c) were raised in two cages, and the EC-GI tumor was transplanted s.c. into 3 to 4 of 5 mice. One or two nude mice were raised as control. When tumor-bearing nude mice began to emaciate, MuIFN-\( \gamma \) or HuIFN-\( \gamma \) (diluted in 200 \( \mu \)l of
0.9% NaCl solution) was injected s.c. into hypercalcemic nude mice at a daily dose of 1 to 20 × 10⁴ units once a day for 5 days. Control tumor-bearing nude mice received only vehicle (0.2 ml of 0.9% NaCl solution). In some experiments, MuIFN-γ (15 × 10⁴ units) was injected s.c. into normal (non-tumor bearing) nude mice once a day for 5 days.

After 1, 3, 5, 7, 10 to 12, and 15 to 20 days, the mice were lightly anesthetized with ether, and blood was taken from the orbita. The serum calcium concentration was determined as described above. Since the tumor growth was not the same and the serum calcium concentrations of tumor-bearing nude mice were between 12 and 24 mg/dl at 7 to 10 wk after transplantation, the experiments were repeated 3 times, using hypercalcemic nude mice with serum calcium levels of 14.0 to 19.3 mg/dl.

Effect of IFN-γ on Urinary Excretion of Calcium. In some experiments, nude mice were housed in metabolic cages (Mabolabo, Sugi-yama-gen, Bunkyo-ku, Tokyo, Japan) between 7 and 8 p.m. Urine was collected at 1 to 2 p.m. on the following day. Throughout these experiments, nude mice were fed sterilized rat chow and water ad libitum. Urinary calcium, phosphate, and creatinine were determined using a multiple autoanalyzer (Mitsubishi-Yuka BCL, Tokyo, Japan).

Histological Examination of Bones and Tumors of Nude Mice. Tumor-bearing nude mice given injections of saline, MuIFN-γ (15 × 10⁴ units), or HuIFN-γ (30 × 10⁴ units) for 5 days were sacrificed and fixed in 10% formalin. The femurs were removed from the mice, decalcified with formic acid-sodium citrate solution, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin.

Histological examination was performed on the femurs and their surrounding epiphyseal cartilage in which osteoclasts are abundant. The number of multinucleated osteoclasts was counted in three 5-μm sections and expressed as an average per mm².

Effect of IFN-γ on Hypercalcemia Induced by PTHrP(1-34) Infusion. PTHrP(1-34) was dissolved in 0.15 M NaCl and 2% cysteine (11). Alzet osmotic pumps (Model 2001; Alza Corp., Palo Alto, CA) were loaded with PTHrP solution to deliver 1.2 μg of PTHrP(1-34) per day. The pumps were s.c. implanted over the back in 14 BALB/c nude mice (9 wk old). Then, MuIFN-γ was injected into 7 mice at a dose of 15 × 10⁴ units once a day for 5 days. Control nude mice (n = 7) received vehicle only (300 μl of saline). After 3 and 5 days, blood was taken from the orbita, and serum calcium concentration was determined as described above.

Effect of IFN-γ on Formation of Osteoclast-like Cells in Vitro. ICR mice or ICR nude mice were given injections of 15 × 10⁴ units of MuIFN-γ for 3 days. Control mice received only vehicle. On the fourth day, the mice were sacrificed, and the effect of IFN-γ on the formation of TRAP-positive, multinucleated cells was determined in vitro, according to the method of Takahashi et al. (12). If brief, bone ends of tibiae removed aseptically were cut off with scissors, and the marrow cavities were flushed out with 1 ml of α-minimal essential medium. The collected marrow cells were washed twice with α-minimal essential medium and cultured in the same medium containing 10% heat-inactivated fetal calf serum (Gibco, Grand Island, NY) at 1.5 × 10⁶ cells/ml in 24-well plates (Corning, Corning, NY; 0.5 ml/well). To stimulate the formation of TRAP-positive, multinucleated cells, 1,25-dihydroxyvitamin D₃ (10⁻⁶ M and 10⁻⁸ M) was added at the beginning of culture and also each time the medium was changed. After 7 days of culture at 37°C under 5% CO₂ and 95% air, cells adhering to the well surface were stained for TRAP, a marker enzyme of osteoclasts, as described elsewhere (12). TRAP-positive, multinucleated cells containing three or more nuclei were counted as osteoclast-like multinucleated cells. All assays were performed in quadruplicate.

Statistical Analysis. Statistical significance was analyzed by the paired or unpaired Student t test. Differences at P < 0.05 were considered significant.

RESULTS

Effect of Calcitonin on Serum Calcium Concentration in Tumor-bearing Nude Mice. When the tumor had grown as large as 1 to 3 cm³, all tumor-bearing nude mice invariably developed marked hypercalcemia (13 to 21 mg/dl). Daily s.c. injection of salmon calcitonin at a dose of 1 unit for 11 days decreased the serum calcium concentration on Days 1 and 3. Thereafter, the serum calcium concentration tended to increase to the pretreatment levels, indicating the development of an escape phenomenon, as demonstrated previously both in vitro and in vivo (Refs. 13 and 14; Fig. 1).

In other experiments, daily s.c. injection of eel calcitonin at a dose of 0.1 unit/day for 17 consecutive days did not decrease the serum calcium level throughout the experimental period (Day 0, 14.1 ± 3.0; Day 1, 13.4 ± 2.0; Day 4, 14.8 ± 2.9; Day
Table 1  Dose-dependent effect of MuIFN-γ on serum calcium concentration

<table>
<thead>
<tr>
<th>Dose of MuIFN-γ (units)</th>
<th>Serum calcium concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (6)</td>
<td>Day 0: 14.8 ± 2.3  Day 7: 15.0 ± 2.6</td>
</tr>
<tr>
<td>1 × 10⁴ (4)</td>
<td>Day 0: 17.0 ± 1.9  Day 7: 18.3 ± 4.5</td>
</tr>
<tr>
<td>5 × 10⁴ (5)</td>
<td>Day 0: 16.6 ± 1.0  Day 7: 13.6 ± 1.7</td>
</tr>
<tr>
<td>20 × 10⁴ (8)</td>
<td>Day 0: 16.7 ± 0.9  Day 7: 11.0 ± 1.6</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number.
* Mean ± SD for 5 to 8 mice.
* P < 0.01.
* *P < 0.001.

7, 16.1 ± 2.8; Day 10, 16.1 ± 3.6; Day 13, 15.8 ± 3.5; Day 17, 14.8 ± 2.4 mg/dl; n = 5; mean ± SD).

Effect of MuIFN-γ on Serum Calcium Concentration of Tumor-bearing Nude Mice. A single injection of MuIFN-γ at a dose of 5 to 20 × 10⁴ units (16.7 to 66.7 µg) did not affect the serum calcium concentration of tumor-bearing nude mice for 5 to 7 days (data not shown). When MuIFN-γ was injected consecutively for 5 days at a dose of 15 to 20 × 10⁴ units (50 to 66.7 µg) into hypercalcemic nude mice, the serum calcium concentration gradually decreased on Days 1 to 5 (Fig. 2). On Day 7, the serum calcium concentration was at a nadir (Day 0, 16.5 ± 1.6 mg/dl; Day 7, 11.2 ± 1.0 mg/dl; mean ± SD; n = 11; P < 0.001). Unlike calcitonin, the effect of MuIFN-γ was more prolonged; i.e., the serum calcium concentration remained low for an additional 5 to 8 days, even after MuIFN-γ injections had been stopped (Day 12.7 ± 1.9 mg/dl; mean ± SD; n = 8; P < 0.01), and then tended to increase again thereafter. Eleven to 15 days after the last MuIFN-γ injection, the serum calcium concentration was increased to the pretreatment level (16.8 ± 2.1 mg/dl; mean ± SD; n = 5). Most of the MuIFN-γ-treated mice survived for more than 1 mo. Blood sampling was stopped in several mice after 10 to 12 days because blindness developed in the early experiments. Two mice died during anesthesia and blood sampling. Three mice were sacrificed on Day 7 for histological studies.

When MuIFN-γ was injected s.c. into normal (non-tumor-bearing) nude mice at a dose of 15 × 10⁴ units for 5 days, the serum calcium concentration did not decrease (Day 0, 10.3 ± 0.2 mg/dl; Day 5, 10.1 ± 0.2 mg/dl; mean ± SD; n = 4).

The serum calcium-lowering effect of MuIFN-γ was dose dependent (Table 1). At a lower dose (1 × 10⁴ units), the serum calcium concentration did not decrease. A significant decrease in serum calcium levels was attained by s.c. injection of 5 × 10⁴ units of MuIFN-γ.

The decrease in the serum calcium concentration was accompanied by a decrease in urinary calcium excretion (Table 2), suggesting that MuIFN-γ was effective on bone but not on kidney.

Table 2  Effect of MuIFN-γ on urinary excretion of calcium and phosphate

<table>
<thead>
<tr>
<th></th>
<th>Calcium (mg)</th>
<th>Phosphate (mg)</th>
<th>Creatinine (mg)</th>
<th>Calcium/creatinine</th>
<th>Phosphate/creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>1.13 ± 0.5*</td>
<td>4.01 ± 1.6</td>
<td>0.53 ± 0.1</td>
<td>2.65 ± 1.2</td>
<td>7.62 ± 0.9</td>
</tr>
<tr>
<td>After</td>
<td>0.30 ± 0.1*</td>
<td>4.21 ± 0.7</td>
<td>0.71 ± 0.1*</td>
<td>0.43 ± 0.2*</td>
<td>6.04 ± 1.5</td>
</tr>
</tbody>
</table>

* Mean ± SD for 5 mice.
* P < 0.05.

Fig. 3. Effect of murine and human IFN-γ on serum calcium concentration in tumor-bearing nude mice. MuIFN-γ (15 × 10⁴ units) or HuIFN-γ (30 × 10⁴ units) was injected s.c. daily for 5 days to three tumor-bearing, hypercalcemic nude mice. On Days 1, 3, 5, and 7, blood was drawn from the orbita, and the serum calcium concentration was determined as described in "Materials and Methods." Points, for three mice given injections of MuIFN-γ (○) or HuIFN-γ (●); bars, SD; arrows, injections of IFN-γ; dotted area, normal range of serum calcium level. Statistical analysis was performed using the Student t test. *, P < 0.05.
INTERFERON AND HYPERCALCEMIA

Fig. 4. Epiphyseal region of the femur of nude mice given injections of saline and MuIFN-γ. Large multinucleated osteoclasts (→) are abundant in the trabecular surface of the femur of a tumor-bearing, hypercalcemic nude mouse treated with saline (left), whereas they are hardly seen in the MuIFN-γ-treated mouse (right). H&E, × 160.

Daily injections of MuIFN-γ (15 × 10⁴ units/day) could not ameliorate the hypercalcemia induced by PTHrP(1-34) infusion at Day 3 (15.6 ± 2.0 mg/dl; mean ± SD; n = 7, P > 0.1) but significantly decreased the serum calcium concentration at Day 5 (12.1 ± 1.0 mg/dl; mean ± SD; n = 7; P < 0.01).

Effect of in Vivo Administration of MuIFN-γ on Osteoclast Formation in Vitro. Since the formation of TRAP-positive osteoclast-like cells in vitro was much better in ICR mice than in BALB/c mice, ICR mice were used in this series of experiments.

Bone marrow cells of control ICR mice developed a significant number of TRAP-positive osteoclast-like cells upon culture with 10⁻⁶ ~ 10⁻⁸ M 1,25-dihydroxyvitamin D₃. Injection of MuIFN-γ at a dose of 15 × 10⁴ units/day for 3 days diminished greatly the formation of TRAP-positive, osteoclast-like cells (Fig. 5, left).

This was also the case in ICR nude mice. Bone marrow cells of control ICR nude mice developed fewer TRAP-positive, osteoclast-like cells than did normal ICR mice. Injection of MuIFN-γ into ICR nude mice for 3 days completely abolished the formation of osteoclast-like cells (Fig. 5, right).

DISCUSSION

The present study has demonstrated that MuIFN-γ, a ubiquitous and long-term inhibitor of bone resorption in vitro (4), produces inhibitory effects on osteoclast formation in vivo, resulting in a substantial decrease of serum calcium concentration in tumor-bearing, hypercalcemic nude mice.

The mechanism by which MuIFN-γ decreased the serum calcium concentration appears to have been inhibition of osteoclastic bone resorption for the following reasons. (a) The decrease in serum calcium concentration was accompanied by a decrease in urinary calcium excretion. (b) In vivo administration of MuIFN-γ to nude mice almost completely abolished the in vitro formation of osteoclast-like cells in mouse marrow cultures. (c) Histological examination revealed a distinct decrease in the number of osteoclasts in the femur of MuIFN-γ-treated mice, whereas no significant tumoricidal effect was detected. IFN-γ is reported to be highly species specific; i.e., murine IFN-γ does not elicit any tumoricidal effect on human cancer cells (5). Conversely, HuIFN-γ does not inhibit bone resorption in fetal mouse forearm bones in vitro (4). This was confirmed in vivo in the present study (Fig. 3). (d) The hypercalcemia of non-tumor-bearing nude mice induced by infusion of PTHrP(1-34) was also ameliorated by repeated injections of MuIFN-γ at Day 5.

Unlike calcitonin, which decreased serum calcium concentration only transiently (on Days 1 to 3), MuIFN-γ produced a gradual and prolonged decrease in hypercalcemic, tumor-bear-

Table 3 Number of multinucleated osteoclasts in the femurs
Saline, MuIFN-γ (15 × 10⁴ units), or HuIFN-γ (30 × 10⁴ units) was injected s.c. daily for 5 days into three tumor-bearing, hypercalcemic nude mice. After sampling of blood from the orbita on day 7, the mice were immersed in 10% formalin and stained as described in “Materials and Methods.” Multinucleated osteoclasts were counted in 3 sections (5 μm) of femur epiphysis. Data are the numbers of multinucleated osteoclasts found in 1 mm² in each section.

<table>
<thead>
<tr>
<th></th>
<th>Section 1</th>
<th>Section 2</th>
<th>Section 3</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Control nude mice</td>
<td>21</td>
<td>14</td>
<td>14</td>
<td>16.3</td>
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<tr>
<td></td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>11.3 ± 2.5</td>
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<tr>
<td></td>
<td>19</td>
<td>14</td>
<td>11</td>
<td>14.6</td>
</tr>
<tr>
<td>Saline-treated, tumor-bearing nude mice</td>
<td>25</td>
<td>40</td>
<td>29</td>
<td>31.3</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>37</td>
<td>36</td>
<td>30.7 ± 3.5</td>
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<tr>
<td></td>
<td>20</td>
<td>30</td>
<td>25</td>
<td>25.0</td>
</tr>
<tr>
<td>HuIFN-γ-treated, tumor-bearing nude mice</td>
<td>20</td>
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<td>30</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>32</td>
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<td>15</td>
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<td>19</td>
<td>30.7</td>
</tr>
<tr>
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<td>2.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.7 ± 0.7</td>
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<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1.3</td>
</tr>
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</table>

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\[ \frac{\text{Number of TRAP-positive cells} \times 10^6}{\text{well}} \]

**Concentration of 1,25-(OH)\(_2\)D\(_3\)**

<table>
<thead>
<tr>
<th>Concentration of 1,25-(OH)(_2)D(_3)</th>
<th>Control-ICR</th>
<th>Nude-ICR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10(^{-9})</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10(^{-8})</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10(^{-7})</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10(^{-6})</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 5. Effect of *in vivo* administration of MuIFN-\(\gamma\) on *in vitro* formation of osteoclast-like cells. MuIFN-\(\gamma\) was injected i.c. at a dose of 15 \times 10^6 units for 3 days into ICR mice (left) or ICR nude mice (right) (6). Control mice received only vehicle (0.2 ml of saline) (C). On the fourth day, the mice were sacrificed, and bone marrow cells were collected. The cells were cultured in 24-multiwell dishes for 6 days as described in "Materials and Methods." Then, the cell monolayers were stained for TRAP, and the TRAP-positive, multinucleated osteoclast-like cells were counted. Points, mean for quadruplicate cultures; bars, SD. A representative result of three independent experiments is shown. *, \(P < 0.01; **, P < 0.001.

\[ 1,25-(OH)\(_2\)D\(_3\), 1,25-dihydroxyvitamin D\(_3\) \]

Effect of MuIFN-\(\gamma\) was very potent, since hypercalcemic, cachectic nude mice were not intensively hydrated during the present experiments; vehicle alone (0.2 ml of saline once a day) did not affect serum calcium levels in the control tumor-bearing nude mice.

It should be stressed that the present findings based on results obtained in T-cell-deficient nude mice cannot be applied directly to patients with malignancy-associated hypercalcemia. In most clinical trials, lower doses of HuIFN-\(\gamma\) (8 to 12 \times 10^6 units/m\(^2\)) are being used (5, 26). Therefore, the doses used in the present experiments (1 to 20 \times 10^6 units/20- to 25-g mouse) may be pharmacological, but comparable to those used for experiments on the tumoricidal effect of MuIFN-\(\gamma\) against xenografts transplanted into nude mice (27). Indeed, nude mice treated with MuIFN-\(\gamma\) seemed less active and ate less food than did the tumor-bearing nude mice treated with anti-PTHrP(1-34) monoclonal antibody (Footnote 5; Ref. 28). Finally, from an economical point of view, IFN-\(\gamma\) would be much more expensive than bisphosphonates, which will be soon available for the treatment of malignancy-associated hypercalcemia in Europe, the United States, and Japan (1).

In summary, MuIFN-\(\gamma\), a potent inhibitor of bone resorption *in vitro*, dose dependently decreased the serum calcium concentration in tumor-bearing, hypercalcemic nude mice for a longer period than did calcitonin. For the treatment of malignancy-associated hypercalcemia, it is essential to develop a new drug like IFN-\(\gamma\), which continuously inhibits bone resorption without the development of an escape phenomenon.

**ACKNOWLEDGMENTS**

We thank Daiichi Pharmaceutical Co. and Shionogi Pharmaceutical Co. for supplying recombinant mouse and human IFN-\(\gamma\), respectively.

**REFERENCES**


11. Rosol, T. J., Capen, C. C., and Horst, R. L. Effects of infusion of human parathyroid hormone-related protein (1-40) in nude mice: histomorphomet-

\[ ^* \text{N. Takahashi, unpublished observation.} \]

\[ ^3 \text{Unpublished observation.} \]


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