ABSTRACT

Cachexia is rare in nude mice bearing human malignant tumors even when the transplanted tumors become as large as the body size of the host. In our series on heterotransplantation of a variety of human malignant tumors into nude mice, a malignant melanoma (SEKI) was found to induce severe body weight loss in the host at the early stage of transplantation. There was no electrolyte disturbance, hyper- or hypoadrenocorticism, hyperthyroidism, or destruction of cells of vital organs to account for the weight loss. Moreover, no evidence was obtained for concomitant infection with bacteria, Mycoplasma or fungi. These cachectic mice revealed remarkably increased levels of serum sialyltransferase and decreased liver catalase activity. The removal of tumor tissues from these mice resulted in prompt recovery of body weight, serum sialyltransferase, and liver catalase activity within 1 to 2 weeks. On the basis of the results obtained, the SEKI melanoma was thought to have produced a pathophysiological state in host nude mice which was very similar to that of cachexia in cancer patients. Nude mice bearing transplants of SEKI melanoma may provide a useful system for the study of cancer cachexia in humans.

INTRODUCTION

Cachexia associated with cancer constitutes one of the most serious problems to clinicians caring for the patients. Little is known concerning the pathophysiology of cachexia, however. Patients with malignant neoplasms suffer from various events which affect their nutrition, such as tumor invasion into vital organs, obstruction of gastrointestinal tracts, and massive hemorrhage. These backgrounds make it difficult to analyze the humoral factor(s) which may induce cachexia per se (3, 5, 25).

Nude mice bearing transplants of human malignant tumors may provide a useful experimental model for the study of cancer cachexia, because metastasis and direct invasion of the transplanted tumor into vital organs or gastrointestinal tracts are rare (6, 7, 9, 20, 26). It has been generally accepted, however, that nude mice bearing human malignant tumors do not develop cachexia even after the transplanted tumor becomes as large as the host itself (6, 20, 26). This may imply that malignant neoplasms do not produce, at least in a large amount, the humoral factors that induce cachexia in the host or that such factors have no effect on nude mice.

In our series on heterotransplantation of human malignant tumors into nude mice, a tissue culture line of a human malignant melanoma, SEKI strain, induced a remarkable loss of body weight in host mice even when the size of the transplanted tumor was small (13). Moreover, the cachexia of these mice disappeared promptly after tumor tissues were extirpated from them.

Account is given in this paper of the similarity between the severe body weight loss of nude mice carrying SEKI melanoma and the cachexia in cancer patients with a special reference to serum sialyltransferase (1, 10, 12, 23) and liver catalase activity (11, 18) and also of a possible production of some humoral factors by the SEKI melanoma.

MATERIALS AND METHODS

Chemicals. CMP-[14C]sialic acid (159 Ci/mol) was purchased from New England Nuclear (Boston, Mass.) and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid was from Calbiochem-Behring Corp. (La Jolla, Calif.). NCS tissue solubilizer was purchased from Amersham/Searle Corp. (Arlington Heights, Ill.). Fetal calf serum was obtained from Sigma Chemical Co. (St. Louis, Mo.). Asialofetuin was prepared from fetuin by mild acid hydrolysis (0.1 N H2SO4, 80°C, 60 min) (2). Titanium sulfate solution (24%) was purchased from Kanto Chemical Co. (Tokyo, Japan). Other chemicals were the purest available from commercial sources.

Nude Mice. Female BALB/c-nu/nu mice distributed at 4 weeks of age by Central Institute for Experimental Animals (Kawasaki, Japan) were maintained in isolation from other experimental animals and kept in cages capped with sterile filter bonnets. Food, water, bedding, and cages were autoclaved before use. Under these conditions, the nu/nu mice remained healthy through the observation period up to 6 months.

Transplantable Human Malignant Tumors. A tissue culture line of a human malignant melanoma, SEKI, established as a suspension culture in 1970 by Dr. M. Shimoyama (National Cancer Center Hospital, Tokyo, Japan) from a tumor in the lower leg skin of a 28-year-old female (22), was supplied through the courtesy of Dr. S. Oboishi of National Cancer Center Institute (14). Cultured melanoma cells (10⁷) were inoculated into the s.c. space of nude mice. Solid tumors developed, and they were serially transferred into the other nude mice. Histology of the transplanted tumors was compatible with amelanotic melanoma. Other human malignant tumors used as controls were a tissue culture line of neuroblastoma (NB-1) (14) and 11 serially transplantable solid tumors including 3 gastric cancers (adenocarcinomas), 2 lung cancers (a squamous cell carcinoma and an oat cell carcinoma), 2 renal cell carcinomas, a lower jaw cancer (squamous cell carcinoma), a penile cancer (squamous cell carcinoma), a choriocarcinoma, and a myeloblastoma. Tumor specimens (5 x 5 x 5 mm) from
Cachexia in Tumor-bearing Nude Mice

malignant tumors were transplanted to the right flank of nude mice by means of a trocar. The tumor size was measured once a week and expressed by the product of 2 dimensions. The body weight of tumor-bearing nude mice was also determined weekly.

**Blood Samples.** About 200 µl of blood were obtained from the postorbital venous plexus of control and tumor-bearing nude mice by a Pasteur pipet once a week. Serum was collected by centrifugation in the cold and immediately tested for sialyltransferase activity by the method described below.

**Tumor Tissues.** Nude mice bearing malignant tumors were sacrificed by decapitation. Tumors were extirpated from the nude mice and freed of skin, connective tissues, and necrotic masses. The tumor tissue was minced with scissors and homogenized in 2 volumes of 0.9% NaCl solution with a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged at 10,000 x g for 15 min, and the supernatant was immediately tested for sialyltransferase activity.

**Assay of Sialyltransferase Activity.** Sialyltransferase activity was determined by a slight modification of the method described by Kessel and Allen (12). The assay mixture consisted of 10 µl of 2% (w/v) asialofetuin, 20 µl of 0.05 M N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (pH 6.5), 20 µl of CMP-[14C]sialic acid (12.6 µM, 159 Ci/mol), and 10 µl of serum or 20 µl of tumor extract as the source of the enzyme. The total volume was adjusted to 100 µl with distilled, deionized water. After incubation at 37° for 30 min, the reaction was terminated by the addition of 1 ml of 0.5 M HCl made 1% with phosphotungstic acid. The resulting precipitate was washed twice with 10% trichloroacetic acid and once with ethanol:ether (2:1) and then solubilized with a NCS tissue solubilizer. Radioactivity of the acid-insoluble material was determined in a liquid scintillation counter and used as a measure of sialyltransferase activity. Incorporation of [14C]sialic acid into asialofetuin was linear when it was plotted against time (0 to 40 min) and enzyme concentration (0 to 40 µl of serum added to the assay mixture).

**Assay of Liver Catalase Activity.** Liver catalase activity was assayed by the method of Nakahara (16). Mice were sacrificed by decapitation. The whole liver was homogenized with a glass homogenizer in 9 volumes of ice-cold deionized water, and the homogenate was diluted 50 times with deionized water (final dilution, 1:500). The reaction mixture contained 0.5 ml of the diluted liver homogenate and 24.5 ml of a substrate solution composed of 5.4 ml of 3% H₂O₂, 7.48 g of Na₂HPO₄·2H₂O, and 3.55 g of KH₂PO₄ in 1 liter of deionized water. After the reaction mixture was mixed and stirred vigorously for 1 min at 0°, a 1-ml portion was pipetted out and mixed with 4 ml of 8% phosphotungstic acid solution. The resulting precipitate was washed with 10% trichloroacetic acid and once with ethanol: ether (2:1) and then solubilized with a NCS tissue solubilizer. Radioactivity of the acid-insoluble material was determined in a liquid scintillation counter and used as a measure of sialyltransferase activity. Incorporation of [14C]sialic acid into asialofetuin was linear when it was plotted against time (0 to 40 min) and enzyme concentration (0 to 40 µl of serum added to the assay mixture).

Liver catalase activity was expressed by the reaction constant (k), calculated as:

\[
k = \frac{1}{\text{Reaction time (sec)}} \times \log_{10} \left( \frac{\text{initial concentration of substrate}}{\text{concentration of substrate after reaction}} \right)
\]

In these experimental conditions, k remained constant at least during the first 2 min of reaction. After 30 min, more than 90% of hydrogen peroxide was consumed and k decreased almost to zero.

**Histological Examinations.** Histological examination was performed on tumor tissues as well as on lung, liver, kidney, and adrenal glands of the host mice. Tissue was fixed in 10% neutral formaldehyde, embedded in paraffin, cut at 8 µm thickness, and stained with hematoxylin and eosin.

**Microbiological Examinations.** Tumor tissues and host nude mice were tested for bacteria, Mycoplasma, and fungi in meat infusion broth, blood agar, thyoglycolate media, pleuropneumonia-like organism agar, and Sabouraud media.

**Serum Concentrations of Thyroxine, Triiodothyronine, Cortisol, and Corticosterone.** Serum levels of thyroxine and triiodothyronine were determined with radioimmunoadsorbent kits of Dainabot Radioisotope Laboratory (Chiba, Japan). Serum levels of cortisol and corticosterone were determined by competitive protein-binding assay in a single extract with methylene chloride followed by separation on a Sephadex LH-20 column (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by Newsome et al. (17).

**RESULTS**

Cachexia in Nude Mice Bearing a Human Malignant Melanoma. Changes in the body weight of nude mice bearing 12 human malignant tumors are shown in Chart 1. For all but 3 tumors, the body weight of host mice increased as tumors grew. Gradual impairment of the host nutrition, however, was
indicated by the lowered rates of body weight increase as tumors grew.

A human malignant melanoma, SEKI, was most remarkable in inducing the loss of weight in host mice (Chart 1a). The body weight of host mice carrying this tumor began to decrease as early as when the tumor size was less than 100 sq mm. Furthermore, progressive decrease of the body weight was observed pari passu with the tumor size. When the tumor was removed from cachectic nude mice bearing the SEKI melanoma, the body weight recovered, and cachectic conditions disappeared within 2 weeks (Figs. 1 and 2). Systemic histological examinations performed while mice were cachectic revealed no pathological findings in lung, liver, kidney, or adrenal glands. Histology of this tumor was compatible with amelanotic melanoma, and necrosis was not as intense as in the other tumors. Microbiological examinations did not reveal bacteria, Mycoplasma, or fungi in the tumor tissues of this melanoma. Only specific-pathogen-free intestinal flora was observed in these cachectic mice. Hematocrit [49.5 ± 1.98% (S.D.)] and serum sodium concentration [155.5 ± 5.3 mEq/liter] of cachectic mice bearing this melanoma were higher, although not significantly, than those of control nude mice (48.2 ± 2.0% and 147.0 ± 7.5 mEq/liter, respectively). Serum levels of calcium and corticosterone did not differ significantly from those in control nude mice, but serum levels of both thyroxine and triiodothyronine were markedly decreased in nude mice carrying SEKI melanoma as compared to those in control nude mice. Cortisol was not detected in sera of both control and SEKI melanoma-bearing mice.

Two other tumors induced the loss of weight in host mice, although to a lesser extent than SEKI melanoma (Chart 1, b and c). In nude mice bearing a lower jaw cancer (Chart 1b), the body weight increased until the tumor size became 150 sq mm, but it decreased thereafter. In mice bearing a lung cancer (I) (Chart 1c), the body weight did not change while the tumor was small, but it decreased when the tumor size became larger than 300 sq mm. These 2 tumors were known to induce remarkable hypercalcemia in host mice with marked calcification in renal tubuli, especially when the tumors became large (19). The weight loss of these host mice appeared only after they developed apparent hypercalcemia.

Sialyltransferase Activity. Sialyltransferase activity in the serum of control nude mice stayed rather constant irrespective of their age (4 to 14 weeks) with considerable small experimental variations (Chart 2). In remarkable contrast, serum sialyltransferase activity in nude mice bearing transplants of SEKI melanoma began to increase shortly after the tumor take and gradually increased as the tumor grew. Removal of the tumor at this point initiated a prompt enzyme activity decrease toward normal levels.

The relationship between serum sialyltransferase activity and tumor size is shown in Chart 3. In SEKI melanoma, the enzyme activity began to elevate even when the tumor size was less than 200 sq mm and rapidly increased as the tumor grew. In mice bearing the other tumors, however, elevation of the en-
zyme activity began only after tumors became considerably large. Levels of sialyltransferase activity in tumor tissues of SEKI melanoma, gastric cancer (H), lower jaw cancer, NB-1, and myeloblastoma were 38.3 ± 3.3, 28.0 ± 2.5, 12.9 ± 0.1, 19.4 ± 2.0, and 56.4 ± 2.4 cpmp per mg protein per 20 min, respectively; no relationship was observed between the sialyltransferase activity in tumor tissues and that in sera of nude mice bearing tumors. In nude mice suffering from wasting disease with hepatitis, serum sialyltransferase activity was also remarkably elevated (582.7 ± 215.8 cpmp/10 μ/20 min).

Table 1 summarizes the results of tumor size, change in body weight, and serum sialyltransferase activity in nude mice at 4 weeks after tumor take, in which tumors were arranged in the same order as that in Chart 1. There was no correlation between tumor size and change in body weight or between tumor size and serum sialyltransferase activity. On the other hand, there was a clear correlation between serum sialyltransferase activity and change in body weight in host nude mice (correlation coefficient, 0.91; p < 0.05).

Liver Catalase Activity. Table 2 shows liver catalase activity in nude mice bearing transplants of various tumors, as well as in controls. In nude mice bearing SEKI melanoma, liver catalase activity was markedly decreased even when the tumors were small, and it returned to the normal level after the removal of tumors. Two of the 3 gastric cancers examined did not affect the liver catalase activity of host mice; especially in nude mice bearing gastric cancer (K), liver catalase activity was not decreased even when the tumor weight became more than 5 g. On the other hand, another gastric cancer (T) caused a marked decrease in the liver catalase activity of host mice when the tumor size was comparable to those of the other 2 gastric cancers. Nude mice bearing these 3 gastric cancers maintained equally good health during these experiments.

Nude mice suffering from wasting disease with hepatitis showed a remarkable decrease in liver catalase activity. Liver catalase activity in BALB/c-μ/μ mice did not differ from that in BALB/c-μ/+ mice. This indicated that liver catalase activity was not affected by the absence of the thymus.

**DISCUSSION**

Loss of body weight is one of the major signs in cancer patients. Many factors have been suggested as the cause of weight loss: taste abnormality (5); energy expenditure (24, 28); disturbed homeostasis of the host metabolism (3, 25); and anorexigenic humoral factors produced by tumors (5, 15, 25). It remains to be determined, however, what factor is essential for the development of cachexia or whether human malignant tumors produce a humoral factor which induces cachexia per se. Lack of suitable experimental models using human malignant tumors seems to have been preventing studies on such problems. In experimental models of animal tumors, some discrepancy between human and animal tumors is inevitable, and loss of total body weight including the tumor is not generally observed when the tumor is small and localized, even though the net body weight without tumor of the host animal begins to decrease in the early stage (24).

In our experiment of heterotransplantation of human malignant tumors into nude mice, total body weight of nude mice with tumors did not usually decrease. However, 3 exceptional tumors [SEKI melanoma, a lower jaw cancer, and a lung cancer (I)], when transplanted, caused a remarkable decrease in the body weight of host mice. Two of them [a lower jaw cancer and a lung cancer (I)] induced severe hypercalcemia in host mice with marked calcium deposition in renal tubuli. Loss of body weight developed in these mice only after the tumor size exceeded a dimension of 300 sq mm, when hypercalcemia became apparent in them. The decrease in body weight in these mice, therefore, might be explained by dehydration and the impairment of renal function, both of which are frequently encountered in patients with severe hypercalcemia. In contrast, body weight of nude mice bearing SEKI melanoma began to decrease as early as when a tumor reached 100 sq mm. The host mice showed no hypercalcemia, tissue damages in vital organs, or evidence of any infections. Furthermore, the cachectic condition was alleviated promptly by removal of the tumor. These results clearly distinguished the cachexia observed in nude mice bearing SEKI melanoma from that in wasting diseases which were often noticed in nude mice bred in conventional environments and attributable to infections. Cachexia associated with SEKI melanoma did not seem to be attributable to the rapid growth rate of the transplanted tumor, because the growth rate of various human malignant tumors did not necessarily correlate to the change in body weight of host mice bearing the tumors (Table 1). All these results pointed to production by this melanoma of humoral factor(s) which induced loss of body weight in the host.
To determine whether cachexia induced by SEKI melanoma was similar to that in cancer patients, we checked some enzymes which are known to be associated with tumor burdens, as well as hemocrit, serum sodium concentration, and some hormones. Results of hemocrit and serum sodium concentration indicated the presence of some dehydration in host mice bearing SEKI melanoma. Hormonal examinations revealed a decrease in serum level of thyroxine and triiodothyronine with normal levels of glucocorticoid hormones in them. The decrease in thyroid hormones might be due to the debilitated state of the host mice; alterations of thyroid hormones have been reported in fasted human subjects (27) and in patients with chronic wasting illness (21).

Elevation of serum sialyltransferase activity was reported in cancer patients (10, 12, 23) and in rats with metastasizing breast cancers (1). Serum sialyltransferase activity increased in parallel with the tumor size and the stage of cancer patients and returned to normal levels after tumor removal (4). In our experiment, serum sialyltransferase activity was most prominently increased in mice bearing the SEKI melanoma and clearly correlated to the tumor size, in contrast with nude mice bearing the other human malignant tumors in which the enzyme activity seemed to increase only when the tumor size became considerably large (Chart 3). Moreover, the removal of SEKI melanoma caused a prompt return of serum sialyltransferase activity to normal level. These results were in good agreement with the previous reports on cancer patients and experimental animals. Our experiment also showed that there was a clear correlation between serum sialyltransferase activity and the change in body weight of host mice bearing transplants of various human malignant tumors (Table 1); the debilitated conditions of host mice induced by tumors seemed to be related to the elevation of the enzyme.

Liver catalase activity markedly decreased in tumor-bearing humans and animals (8, 11, 18) and also in several conditions such as starvation and inanition (11). In our study, liver catalase activity was remarkably decreased in nude mice bearing SEKI melanoma and a gastric cancer (T) as well as those suffering from wasting disease with hepatitis (Table 2). Although it was not determined whether the marked decrease in nude mice bearing SEKI melanoma was due to the tumor burden itself or secondary to the cachectic condition, the change in liver catalase activity bore a striking resemblance to that in cancer patients.

On the basis of the results reported herein, the cachectic condition in nude mice bearing the SEKI melanoma closely resembled that in cancer patients. Indeed, the SEKI melanoma-nude mouse system may provide a useful model for cancer cachexia in humans, especially for the investigation of cachexia-producing humoral factor(s) elaborated by tumors.

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Yukio Kondo, Kanji Sato, Yoshito Ueyama, et al.


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