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Characterization of Mice Bearing Subclones of Colon 26 Adenocarcinoma Disqualifies Interleukin-6 as the Sole Inducer of Cachexia

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A subclone (clone 20) of chemically induced, murine colon adenocarcinoma with a potent ability to induce cachexia and another subclone (clone 5) without such an activity were transplanted to syngeneic mice (CDF_1) and their tissue weights, blood components and cytokine levels in sera were compared. Mice transplanted with clone 20 showed a profound body-weight loss by 15 days after inoculation when the tumor accounted for less than 1% of the body weight, along with marked reduction of food and water intakes. Thereafter, they transiently gained in body weight with restoration of food and water intakes. Thus, the change in body weight was biphasic and not proportional to the tumor size. Body fat was lost preferentially, accompanied with a decrease in plasma triglyceride levels. The thymus contracted remarkably, and the peripheral lymphocyte count decreased extensively. Mice transplanted with clone 5, in contrast, did not show any of these changes characteristic of cachexia. Serum concentration of interleukin-6, which has been proposed as the principal inducer of cachexia in mice with colon 26, increased in mice with clone 5 to levels comparable to those in mice with clone 20. The changes in mice bearing clone 20 could not all be explained in terms of known biological activities of interleukin-6. Additional unknown factors, therefore, are presumed to contribute to cachexia in mice with clone 20. Identification of them should be helpful in the care of cachectic patients.

Key words: Cancer cachexia — Colon 26 adenocarcinoma — Interleukin-6 — Interleukin-1 — Tumor necrosis factor

Cachexia, an exhaustive state with severe weight loss, is a serious problem in cancer patients affecting their morbidity and mortality. It decreases their quality of life and shortens their life-span.[1,2] It is therefore desirable to elucidate the mechanism of cancer cachexia in order to develop effective measures to prevent or reverse it.

Animal tumors are helpful for the study of cachexia, because they provide an opportunity to examine the sequellae of cachexia on a total body scale. Most animal tumors typified by rodent tumors,[3] however, do not induce cachexia in hosts until the tumor occupies as much as 10–30% of the body weight.[4,5] This sharply contrasts with human tumors, that rarely exceed 5% of the body weight even at the end-stage.[6,7] Nude mice bearing human tumors would not reflect exactly the events in cachexia, either, because of a disproportionate tumor mass or immune defects not seen in cancer patients.[8–10]

Colon 26 is a chemically induced, murine colon-adenocarcinoma cell line known to induce marked cachexia in mice.[11,12] This tumor is transplantable to healthy syngeneic mice with normal immune function, and induces severe cachexia before it grows extensively. Thus, mice bearing colon 26 closely resemble human patients with cachexia, in whom massive tumor growth is not necessarily observed.

Several studies have been reported on colon 26, focusing on cytokines[13,14] and arachidonate derivatives[15] as possible endogenous mediators of cachexia. A full understanding of cachexia will not be achieved, however, until the alterations in the entire body are elucidated.

In order to put cachexia into a wider perspective, we examined the metabolic and tissue changes in mice inoculated with two subclones of colon 26, clone 20 with a potent cachexia-inducing capacity and clone 5 without such an activity.

MATERIALS AND METHODS

Animals Male CDF_1 (BALB/c × DBA/2 F_1) mice aged 6–8 weeks were from Charles River Japan (Atsugi, Kanagawa). They were housed in individual glass metabolic cages in a completely closed system (Sugiyama-Gen, Tokyo). Mice were fed *ad libitum* on Rodent laboratory chow (Nippon Bio-Supp., Tokyo) throughout experiments. Consumptions of food and water, as well as amounts of feces and urine, were recorded at 10:00 a.m. daily. Body weight and tumor size were determined three times a week. Tumor size was measured in terms of two diameters, the greatest diameter (a) and lesser diameter (b), positioned at right angles to each other. The tumor

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weight was estimated by using the formula $ab^2/2 \times F$, where F is a correction factor. The correction factor of 1 was obtained by comparing actual tumor weight (g) with tumor volume ($ab^2/2$) in preparative experiments.

Cells Colon 26, a murine colon-adenocarcinoma cell line and its subclone (clone 20), both with a potent cachexia-inducing capacity, were propagated and generously provided by Nippon Roche Research Center (Kamakura, Kanagawa). Another subclone (clone 5), also established and donated by Nippon Roche Research Center, grows in a manner similar to the parent colon 26 cell line both *in vivo* and *in vitro*, but does not induce severe cachexia. Cells were placed in wells of a culture plate (96-well, Corning, NY) at a density of 10^4 cells/ml in 200 μl of RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (GIBCO, Grand Island, NY), and grown in a humidified atmosphere containing 5% (v/v) CO_2. Cells in seven wells each were counted at 21, 37, 46, 56, 66 and 83 h after plating. Conditioned medium was collected after 83 h of cell culture, and was stored at -80°C until assay for cytokines.

Tumor inoculation Cells of clone 20 and clone 5 were implanted in Corning plastic culture flasks (75 cm^2, Iwaki Glass, Tokyo) at a density of 10^4 cells/cm^2 in 20 ml of RPMI 1640 medium supplemented with 10% FBS. Cells were grown till they became subconfluent. Then they were trypsinized and suspended in Hanks' balanced salt solution (GIBCO) at a density of 5×10^6 cells/ml. A cell suspension (0.2 ml) containing 10^6 cells was inoculated subcutaneously into the flank of mice.

Blood sampling and organ extirpation Blood samples were collected from mice by cardiac puncture at the right atrium with a 25-gauge needle, after thoracotomy under anesthesia with pentobarbital (60 μg/g body weight), on the day before, as well as the 11th and 16th days after inoculation of clone 20. Blood samples from mice inoculated with clone 5 were collected in the same way on the 16th and 21st days after they had received the tumor. Blood cells were counted by a hematology analyzer (Technicon H-1, Technicon Instruments Corporation, Tarrytown, NY) adjusted for murine blood cells. The hemogram was examined on blood smears stained with Giemsa-Wright. Serum levels of total protein, albumin, cholesterol, triglyceride, non-esterified fatty acid and sialic acid were determined by a clinical auto-analyzer (Clinalyzer JCA-RX20, JEOL Ltd., Tokyo). Liver, spleen, thymus, epididymal fat-pad and gastrocnemius muscle, as well as transplanted tumor, were extirpated and weighed in the wet condition.

Cytokine determination Blood was centrifuged, and serum was collected and stored at -80°C until assay for cytokines. Levels of tumor necrosis factor (TNF), interleukin-6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in serum and culture medium were measured by enzyme-linked immunosorbent assays, using Factor-test mTNF-α (Genzyme, Cambridge, MA), murine IL-6 and GM-CSF ELISA kits (Endogen, Boston, MA), respectively.

Statistical analysis Statistic analysis was performed using Student's t test.

RESULTS

Body weight and survival length The body weight of mice inoculated with clone 20 started to decrease rapidly around 10 days after tumor inoculation. Mice developed full-blown cachexia with decreased activity and napped hair. The decrease in body weight continued during the following 5 to 6 days, reaching 35% of the pre-inoculation value (Fig. 1). This rapid loss of body weight paralleled marked reductions in food and water intakes. Urine volume and feces weight decreased in a similar manner until 18 days after inoculation (Fig. 2).

Seven (64%) of 11 mice bearing clone 20 died of cachexia accompanied by a rapid decline in body weight by the 20th day after tumor inoculation, even though the weight of tumor at death barely exceeded 0.3 g (corresponding to 1% of body weight). When mice survived this rapid weight loss, however, they always recovered their appetite during the following several days, with a gradual gain in the body weight. After several days of this recovery, the mice lost appetite and body weight again. As a consequence, body weight waxed and waned

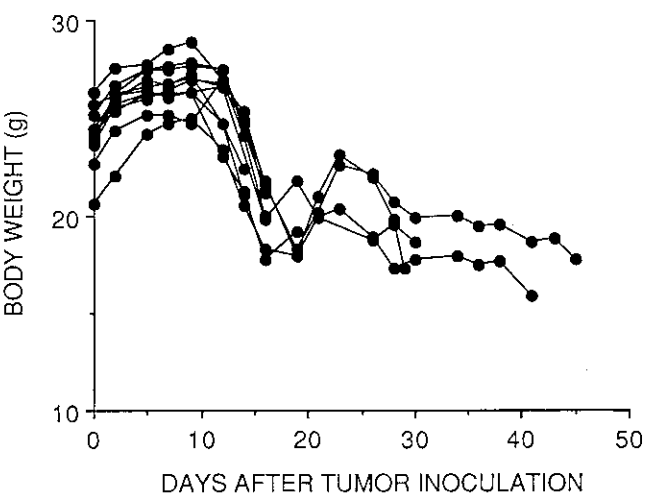

Fig. 1. Body weights of CDF_1 mice with clone 20 (a subclone of colon 26). Cells were trypsinized and suspended in Hanks' balanced salt solution at a density of 5×10^6/ml. A suspension (0.2 ml) containing 10^6 cells was inoculated subcutaneously into the flank of mice. Body weights were measured three times a week.

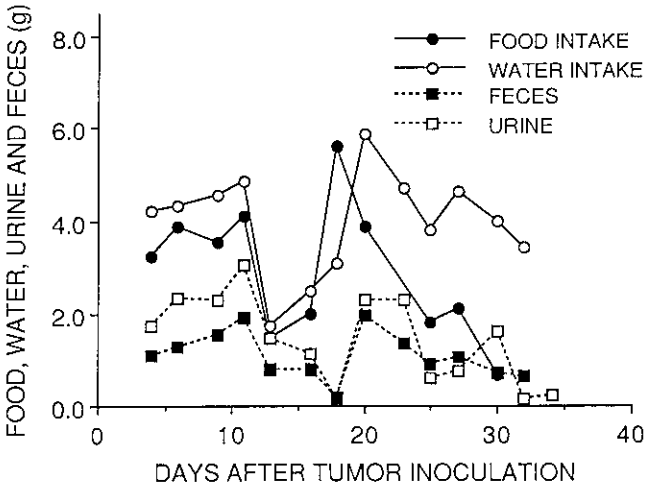

Fig. 2. Food and water intakes and volumes of feces and urine of the mice after inoculation of clone 20. Tumor-bearing mice were housed individually in closed metabolic cages after inoculation. Food and water intakes were calculated daily by subtracting residuals from the initial amount. Feces and urine were collected in a completely closed system and measured daily. Data from one mouse examined three times a week were plotted.

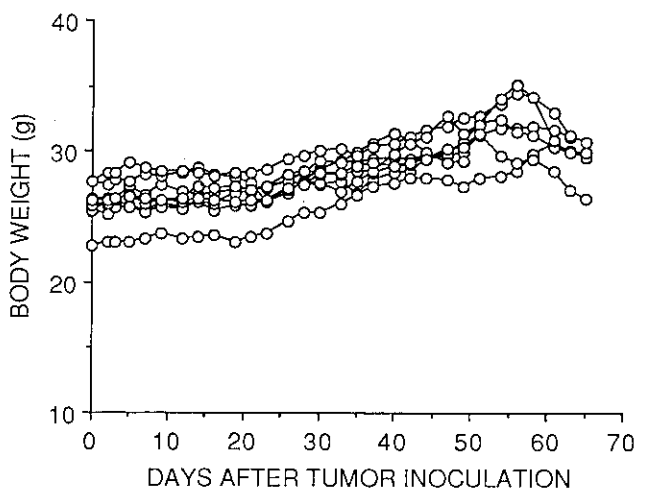

Fig. 3. Body weights of CDF_1 mice with clone 5 (a subclone of colon 26). Cells were cultured and inoculated in mice as described in the legend to Fig. 1. Body weights were measured three times a week.

in a characteristic biphasic pattern (Fig. 1). The temporary weight gain was not due to increase in tumor size, since the biphasic weight change was still seen after the tumor weight was subtracted from the body weight.

In mice with clone 5, the body weight, food and water intakes, feces weight and urine volume did not decrease

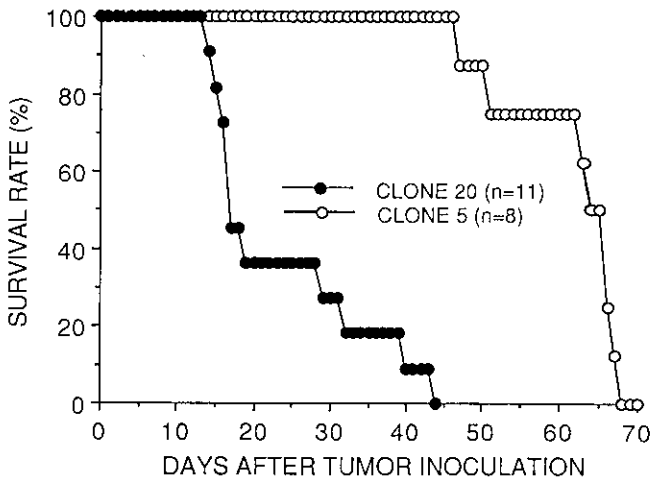

Fig. 4. Effects of tumor inoculation on survival rates. Eleven mice were inoculated with 10^6 cells of clone 20, and eight mice with an equal number of clone 5 cells.

significantly until the animals died at 60 to 70 days after tumor inoculation (Fig. 3). They did not exhibit a cachectic appearance, and lived longer than mice with clone 20 (Fig. 4). At the time of death, many metastatic lesions were observed in both lungs and liver of all mice with clone 5, in sharp contrast to the lack of such metastases in mice with clone 20. The growth rate of clone 20 tumor was always slower than that of clone 5 tumor *in vivo*, though clone 20 cells replicated more rapidly than clone 5 cells *in vitro* (Fig. 5).

Among various body components weighed, fat showed a remarkably rapid depletion (Table I). The weight of the epididymal fat-pad in mice bearing clone 20 decreased rapidly, *pari passu* with the decline of body weight, while that in mice with clone 5 decreased steadily but very slowly. The depletion of fat in mice with clone 20 continued even during a temporary recovery of body weight around 15 days after inoculation, and therefore, was not simply ascribable to decreased calorie intake. Such a rapid depletion of body fat was not observed in normal mice fed with amounts of laboratory chow equal to those consumed by tumor-bearing mice (data not shown).

Changes in the thymus of mice with clone 20 were remarkable (Table I). The weight of the thymus in mice with clone 20 (6.5 ± 2.1 g) was much lower than that in mice with clone 5 (25.2 ± 2.7 g) ($P < 0.01$). Muscle consistently decreased in weight, but at a much slower rate than body weight or adipose tissue. Again, these changes were not conspicuous in mice with clone 5, except for liver and spleen, which increased in weight comparably to those in mice with clone 20 (Table I).

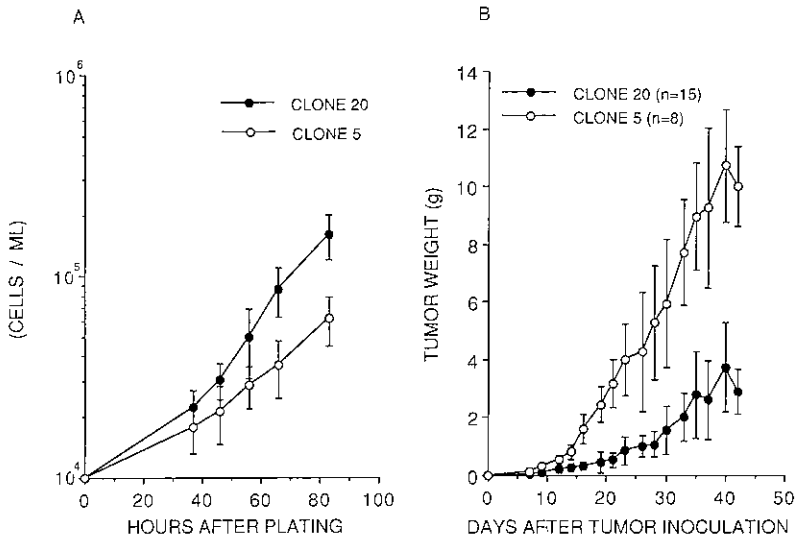

Fig. 5. A: Growth of clone 20 and clone 5 cells *in vitro*. Cells of either clone were plated in wells. Numbers in seven wells were counted for each clone. B: Growth of clone 20 and clone 5 tumors *in vivo*. Cells of either clone were inoculated into mice as described in the legend to Fig. 1. Tumor weights were estimated from two perpendicular diameters (see "Materials and Methods").

Table I. Tissue and Organ Weights of Mice Bearing Subclones of Colon 26[a)]

Tumor & days after inoculation	Epididymal fat pad	Muscle	Thymus	Liver	Spleen
Day 0 (n=5)	280±71	118±15	32.7±1.1	1,142±117	98±11
Clone 20					
Day 11 (n=5)	164±43	98±28	23.4±7.3	1,118±77	156±22[c)]
Day 16 (n=9)	42±31[b)]	89±9[c)]	6.5±2.1[b)]	1,016±186	133±36
Clone 5					
Day 16 (n=5)	302±33	106±11[c)]	25.2±2.7	1,220±20	180±22[c)]
Day 21 (n=6)	192±58	103±20	20.0±4.1	1,368±89	328±74[d)]

a) Data represent the mean±SD in mg.
b) Significantly different ($P<0.01$) from the values for day 0 and day 11 of clone 20, and from the values for day 16 and day 21 of clone 5.
c) Significantly different from the value for day 0 at $P<0.05$.
d) Significantly different from the value for day 0 at $P<0.01$.

Blood cells and serum biochemistry Red blood cell counts and hemoglobin concentrations in mice with clone 20 decreased gradually as cachexia progressed, contrasting with white blood cell and platelet counts, which increased markedly (Table II). Notably, lymphocyte counts in the peripheral blood decreased rapidly in mice with clone 20. Changes in lymphocyte counts appeared to reflect the thymus weight; they decreased concurrently with initial body-weight loss. Although similar changes in blood cells were observed in mice with clone 5, the decline in lymphocyte count was not observed in them (Table II). Serum protein (9.1±1.0 g/100 ml on day 0 vs. 6.1±1.5 g/100 ml on day 16: n=3), albumin (2.2±0.2 g/100 ml on day 0 vs. 1.1±0.2 g/100 ml on day 16: n=3) and triglycerides (153±88 g/100 ml on day 0 vs. 91±61 on day 16: n=3) decreased, while sialic acid, an acute-phase reactant, increased in mice with clone 20 (60±8 g/100 ml on day 0 vs. 126±43 g/100 ml on day 16: n=3).

Cytokine levels in conditioned media and murine sera TNF concentrations in conditioned media from the culture either of clone 20 or clone 5 cells were below detectable levels (less than 50 pg/ml). IL-6 was detectable in conditioned media of clone 20 (445±150 pg/ml: n=5) and clone 5 (215±165 pg/ml: n=5). GM-CSF was detected in culture media of both clones and sera of mice inoculated with either clone (data not shown). Serum concentrations of TNF in mice inoculated with either clone 20 or clone 5 were below detectable levels (less than 50 pg/ml). Serum levels of IL-6 in mice bearing clone 20 on the 11th and 16th days after tumor inoculation were elevated to 196±53 pg/ml (n=5) and

Table II. Hemogram of Mice Bearing Colon 26[a]

Tumor & days after inoculation	RBC ($\times 10^4$/mm^3)	Hb (g/dl)	WBC (/mm^3)	Lymphocytes (/mm^3)	Platelets ($\times 10^3$/mm^3)
Day 0 (n=6)	1,038±25	15.0±0.3	2,888±796	1,827±807	756±308
Clone 20					
Day 11 (n=5)	996±32[c]	14.3±0.6	4,752±2,328	902±378	2,108±116[b]
Day 16 (n=7)	980±43[b]	14.3±0.7[c]	5,380±1,774[c]	766±241[c,d]	2,039±247[b]
Clone 5					
Day 16 (n=5)	960±55[c]	14.1±0.4[c]	4,590±351[c]	1,545±482	1,635±100[b]
Day 21 (n=4)	881±24[b]	12.2±0.3[b]	9,380±1,622[b]	2,065±194	1,849±117[c]

a) Data represent the mean±SD for red blood cells (RBC), hemoglobin (Hb), white blood cells (WBC), lymphocytes and platelets.
b) Significantly different from the value for day 0 at $P<0.01$.
c) Significantly different from the value for day 0 at $P<0.05$.
d) Significantly different ($P<0.05$) from the values for day 16 and day 21 of clone 5.

Table III. IL-6 in Culture Media and Sera of Mice[a]

Sample	Clone 20	Clone 5
Conditioned medium (84 h after plating)	445±150 (n=5)	215±165 (n=5)
Serum		
Day 11	196±53 (n=5)	—
Day 16	308±134 (n=5)	225±76 (n=6)
Day 21	—	287±119 (n=5)

a) IL-6 concentrations in conditioned medium and murine sera were measured by enzyme-linked immunosorbent assay. Data represent the mean±SD in pg/ml.

308±134 pg/ml (n=5), respectively. IL-6 concentrations in serum of mice inoculated with clone 5 also increased to 225±76 pg/ml (n=6) and 287±119 pg/ml (n=5) on the 16th and 21st days after tumor inoculation, respectively (Table III).

DISCUSSION

The mechanism of cancer cachexia is extremely complex, but a decade ago, critical roles were established for several endogenous mediators in the development of cachexia.[16-18] Recently, an endogenous mediator, IL-6, has been implicated in the cancer cachexia of mice bearing a subclone of clone 26.[13] Information on changes of tissues and blood components of mice bearing this tumor, however, is far from being complete. Cachexia is a consequence of many interrelated metabolic disorders, and is unlikely to involve only a single cytokine. These considerations underscore the need for close observation of all the changes occurring in tumor-bearing animals *in vivo*.

Among tissues and organs examined, the most remarkable change was a rapid and extensive depletion of fat in adipose tissue, contrasting with the decrease of lean body mass, which was slow and much smaller in extent. Marked contraction of the thymus and prominent enlargement of the spleen were among the other noteworthy changes. Taken together with the significant decrease in peripheral lymphocytes, these changes suggest that active immune responses would have been maintained until very late after the start of cachexia. Uncompromised immune responses of hosts, indeed, would have been responsible for the inhibited *in vivo* growth of the clone 20 tumor.

An additional observation of note was that the cachexia induced by clone 20 tumor was not ascribable to tumor invasion or metastasis. This view was supported by the finding that another subclone of the same origin (clone 5) did not induce obvious cachectic changes even after it grew as big as, or metastasized even more extensively than, clone 20 (unpublished observations).

Anorexia is one of the important mechanisms of cachexia.[19] As in other models,[3] the cachexia induced by clone 20 developed in parallel with a profound reduction in food and water intakes starting around 10 days after the inoculation of tumor cells. Characteristic changes of cachexia induced by this tumor, highlighted by a rapid and profound decrease of fat tissues to the extent that the epididymal fat-pads disappeared, however, were not observed in control pair-fed mice. The wasting of tumor-bearing mice, therefore, was not attributable to a negative calorie balance due to reduced food intakes.

Strassmann *et al.*[13] have ascribed the cachexia induced by colon 26 to IL-6 produced by this tumor. This suggestion is inconsistent with the lack of serious wasting leading to cachexia in transgenic mice expressing IL-6 or in patients with IL-6-producing tumors, at least during an early stage of tumor growth.[20] Unlike mice with clone

20, animals in the cited studies did not show reduced food and water intakes. We observed a marked increase of IL-6 concentration in sera from mice inoculated with cachexia-inducing clone 20. Serum IL-6 increased to similar levels in mice bearing clone 5, however, even though they did not exhibit a cachectic appearance or body-weight loss for a long time. Moreover, IL-6 by itself did not display lipolytic activity in cultured, fully differentiated 3T3-L1 adipocytes (unpublished observations).

Increase of white blood cell and platelet counts in mice either with clone 20 or clone 5 could be explained by the production of IL-6 and GM-CSF by either clone 20 or clone 5 cells *in vitro*. This view is supported by results obtained with IL-6-treated mice, in which megakaryocytes mature and platelets increase in number with few adverse effects.[20] Rapid and marked contraction of the thymus as well as decrease of peripheral lymphocytes, which were not observed in mice with non-cachexia-inducing clone 5, suggest the involvement of T-cell-mediated immune responses in mice with cachexia-inducing clone 20. Again, these immunological changes are not consistent with results in transgenic mice expressing IL-6, which demonstrate marked plasmacytosis and massive plasma-cell infiltration in the thymus.[21] Some additional factors seem to be necessary, therefore, for inducing cachexia in mice carrying clone 20 of colon 26.

Up to the present, several well-characterized factors, including cachectin/TNF,[22–26] IL-1,[5, 27, 28] interferon-γ[29] and leukemia inhibitory factor,[30, 31] have been proposed as cachexia-inducing principles. Additional factors such as toxohormone-L,[17] lipid-mobilizing factor[32] and lipolytic factor[18] have also been proposed; their amino acid sequences remain to be determined. The changes demonstrated in this study in cachectic mice with clone 20 suggest that not only IL-6, but also some of the above factors contribute to cachexia. GM-CSF was detected in serum of the tumor-bearing mice and in conditioned media from the culture with insignificant differences between clone 20 and clone 5. Trials to reverse the cachexia by neutralizing cachectin/TNF or IL-1 with anti-mouse-cachectin/TNF or IL-1 receptor antagonist were conducted, but little, if any effect was seen (unpublished observation). The nature of the humoral factor(s) inducing cachexia still remains unclear.

The biphasic body-weight change, accompanied with a temporary recovery of food and water intakes, is not seen in other models of cachexia. The mechanism of this phenomenon is not clear either. Tachyphylaxis in animals receiving repeated injections of cachectin/TNF might be relevant,[29, 33] although the role of cachectin/TNF in inducing cachexia remains dubious.

The results reported herein complement those in previous reports on this animal model of cachexia induced by colon 26, as well as its subclone (clone 20). Accumulation of data on total body changes in this model, as attempted in the present study, should be helpful in improving the treatment of patients with cancer cachexia.

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REFERENCES

1) DeWys, W. D., Begg, D., Lavin, P. T., Band, P. R., Bennett, J. M., Bertino, J. R., Cohen, M. H., Douglas, H. O., Engstrom, P. F., Ezdinli, E. Z., Horton, J., Johnson, G. J., Moertel, C. G., Oken, M. M., Perlia, C., Rosenbaum, C., Silverstein, M. N., Skeel, R. T., Sponzo, R. W. and Tormay, D. C. Prognostic effect of weight loss prior to chemotherapy in cancer patients. *Am. J. Med.*, **69**, 491–497 (1980).
2) Warren, S. The immediate causes of death in cancer. *Am. J. Med. Sci.*, **184**, 610–615 (1932).
3) Garattini, S. and Guaitani, A. Animal models for the study of cancer-induced anorexia. *Cancer Treat. Rep.*, **65** (Suppl. 5), 23–35 (1981).
4) Strain, A. J., Easty, G. C. and Neville, A. M. A new experimental model of human cachexia. *Invest. Cell Pathol.*, **2**, 87–96 (1979).
5) Gelin, J., Moldawer, L. L., Lönnroth, C., Sherry, B., Chizzonite, R. and Lundoholm, K. Role of endogenous tumor necrosis factor α and interleukin 1 for experimental tumor growth and the development of cancer cachexia. *Cancer Res.*, **51**, 415–421 (1991).
6) Nathanson, L. and Hall, T. C. A spectrum of tumors that produce paraneoplastic syndromes. Lung tumors: how they produce their symptomes. *Ann. N.Y. Acad. Sci.*, **230**, 367–377 (1974).
7) Norton, J. A., Peacock, J. L. and Morrison, S. D. Cancer cachexia, *Crit. Rev. Oncol. Hematol.*, **7**, 289–327 (1987).
8) Kondo, Y., Sato, K., Ueyama, Y. and Ohsawa, N. Serum sialyltransferase and liver catalase activity in cachectic nude mice bearing a human malignant melanoma. *Cancer Res.*, **41**, 2912–2916 (1981).
9) Black, K., Garrett, I. R. and Mundy, G. R. Chinese

hamster ovarian cells transfected with the murine interleukin-6 gene cause hypercalcemia as well as cachexia, leukocytosis and thrombocytosis in tumor-bearing nude mice. *Endocrinology,* **128,** 2657–2659 (1991).
10) Yoneda, T., Alsina, M. A., Chavez, J. B., Bonewald, L., Nishimura, R. and Mundy, G. R. Evidence that tumor necrosis factor plays a pathogenetic role in the paraneoplastic syndromes of cachexia, hypercalcemia, and leukocytosis in a human tumor in nude mice. *J. Clin. Invest.,* **87,** 977–985 (1991).
11) Tanaka, Y., Eda, H., Tanaka, T., Udagawa, T., Ishikawa, T., Horii, I., Ishitsuka, H., Kataoka, T. and Taguchi, T. Experimental cancer cachexia induced by transplantable colon 26 adenocarcinoma in mice. *Cancer Res.,* **50,** 2290–2295 (1990).
12) Corbett, T. H., Griswold, D. P., Jr., Roberts, B. J., Peckham, J. C. and Schabel, F. M., Jr. Tumor induction relationships in development of transplantable cancers of the colon in mice for chemotherapy assays, with a note on carcinogen structure. *Cancer Res.,* **35,** 2434–2439 (1975).
13) Strassmann, G., Fong, M., Kenney, J. S. and Jacob, C. O. Evidence for the involvement of interleukin 6 in experimental cancer cachexia. *J. Clin. Invest.,* **89,** 1681–1684 (1992).
14) Strassmann, G., Jacob, C. O., Evans, R., Beall, D. and Fong, M. Mechanisms of experimental cancer cachexia: interaction between mononuclear phagocytes and colon-26 carcinoma and its relevance to IL-6-mediated cancer cachexia. *J. Immunol.,* **148,** 3674–3678 (1992).
15) Tanaka, Y., Tanaka, T. and Ishitsuka, H. Antitumor activity of indomethacin in mice bearing advanced colon 26 carcinoma compared with those with early transplants. *Cancer Res.,* **49,** 5935–5939 (1989).
16) Kawakami, M., Ikeda, Y., Le-Trang, N., Vine, W. and Cerami, A. Studies of conditions and agents that stimulate and inhibit the production of cachectin by macrophages. *In* "Proceedings of IUPHAR Vol. 2," ed. W. Paton, pp. 377–384 (1984). Macmillan Press, London.
17) Masuno, H., Yoshimura, H., Ogawa, N. and Okuda, H. Isolation of a lipolytic factor (toxohormone-L) from ascites fluid of patients with hepatoma and its effect on feeding behavior. *Eur. J. Cancer Clin. Oncol.,* **20,** 1177–1185 (1984).
18) Beck, S. A. and Tisdale, M. J. Production of lipolytic and proteolytic factors by a murine tumor-producing cachexia in the host. *Cancer Res.,* **47,** 5919–5923 (1987).
19) Bruera, E. and MacDonald, R. N. Nutrition in cancer patients: an update and review of our experience. *J. Pain Symptom Manage.,* **3,** 133–140 (1988).
20) Ishibashi, T., Shikama, Y., Kimura, H., Kawaguchi, M., Uchida, T., Yamamoto, T., Okano, A., Akiyama, Y., Hirano, T., Kishimoto, T. and Maruyama, Y. Thrombopoietic effects of interleukin-6 in long-term administration in mice. *Exp. Hematol.,* **21,** 640–646 (1993).
21) Hirano, T., Akira, S., Taga, T. and Kishimoto, T. Biological and clinical aspects of interleukin 6. *Immunol. Today,* **11,** 443–449 (1990).
22) Kawakami, M. and Cerami, A. Studies of endotoxin induced decrease in lipoprotein lipase activity. *J. Exp. Med.,* **154,** 631–639 (1981).
23) Kawakami, M., Pekala, P. H., Lane, M. D. and Cerami, A. Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cell. *Proc. Natl. Acad. Sci. USA,* **79,** 912–916 (1982).
24) Kawakami, M., Murase, T., Ogawa, H., Ishibashi, S., Mori, N., Takaku, F. and Shibata, S. Human recombinant TNF suppresses lipoprotein lipase activity and stimulates lipolysis in 3T3-L1 cells. *J. Biochem.,* **101,** 331–338 (1987).
25) Kawakami, M., Watanabe, N., Ogawa, H., Kato, A., Sando, H., Yamada, N., Murase, T., Takaku, F., Shibata, S. and Oda, T. Cachectin/TNF kills or inhibits the differentiation of 3T3-L1 cells according to developmental stage. *J. Cell. Physiol.,* **138,** 1–7 (1989).
26) Oliff, A., Jones, D. D., Boyer, M., Martinez, D., Kiefer, D., Vuocolo, G., Wolfe, A. and Socher, S. H. Tumors secreting human TNF/cachectin induce cachexia in mice. *Cell,* **50,** 555–563 (1987).
27) Ogawa, H., Nielsen, S. and Kawakami, M. Cachectin/tumor necrosis factor and interleukin-1 show different modes of combined effect on lipoprotein lipase activity and intracellular lipolysis in 3T3-L1 cells. *Biochem. Biophys. Acta,* **1003,** 131–135 (1989).
28) Kawakami, M., Watanabe, N., Ogawa, H., Murase, T., Yamada, N., Sando, H., Shibata, S., Oda, T. and Takaku, F. Specificity in metabolic effects of cachectin/TNF and other related cytokines. *Ann. N.Y. Acad. Sci.,* **587,** 339–350 (1990).
29) Langstein, H. N., Doherty, G. M., Fraker, D. L., Buresh, C. M. and Norton, J. A. The roles of γ-interferon and tumor necrosis factor α in an experimental rat model of cancer cachexia. *Cancer Res.,* **51,** 2302–2306 (1991).
30) Mori, M., Yamaguchi, K. and Abe, K. Purification of a lipoprotein lipase-inhibiting protein produced by a melanoma cell line associated with cancer cachexia. *Biochem. Biophys. Res. Commun.,* **160,** 1085–1092 (1989).
31) Kawakami, M., Kondo, Y., Imai, Y., Hashiguchi, M., Ogawa, H., Hiragun, A., Aotsuka, S., Shibata, S., Oda, T., Murase, T. and Takaku, F. Suppression of lipoprotein lipase in 3T3-L1 cells by a mediator produced by SEKI melanoma, a cachexia-inducing human melanoma cell line. *J. Biochem.,* **109,** 78–82 (1991).
32) Kitada, S., Hays, E. F. and Mead, J. F. Characterization of a lipid mobilizing factor from tumors. *Prog. Lipid Res.,* **28,** 823–826 (1981).
33) Socher, S. H., Friedman, A. and Martinez, D. Recombinant human tumor necrosis factor induces acute reductions in food intake and body weight in mice. *J. Exp. Med.,* **167,** 1957–1962 (1988).