Jpn. J. Cancer Res. 88, 184–190, February 1997

Bovine Lactoferrin and Lactoferricin, a Peptide Derived from Bovine Lactoferrin, Inhibit Tumor Metastasis in Mice

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We investigated the effect of a bovine milk protein, lactoferrin (LF-B), and a pepsin-generated peptide of LF-B, lactoferricin (Lfcin-B), on inhibition of tumor metastasis produced by highly metastatic murine tumor cells, B16-BL6 melanoma and L5178Y-ML25 lymphoma cells, using experimental and spontaneous metastasis models in syngeneic mice. The subcutaneous (s.c.) administration of bovine apo-lactoferrin (apo-LF-B, 1 mg/mouse) and Lfcin-B (0.5 mg/mouse) 1 day after tumor inoculation significantly inhibited liver and lung metastasis of L5178Y-ML25 cells. However, human apo-lactoferrin (apo-LF-H) and bovine holo-lactoferrin (holo-LF-B) at the dose of 1 mg/mouse failed to inhibit tumor metastasis of L5178Y-ML25 cells. Similarly, the s.c. administration of apo-LF-B as well as Lfcin-B, but not apo-LF-H and holo-LF-B, 1 day after tumor inoculation resulted in significant inhibition of lung metastasis of B16-BL6 cells in an experimental metastasis model. Furthermore, in *in vivo* analysis for tumor-induced angiogenesis, both apo-LF-B and Lfcin-B inhibited the number of tumor-induced blood vessels and suppressed tumor growth on day 8 after tumor inoculation. However, in a long-term analysis of tumor growth for up to 21 days after tumor inoculation, single administration of apo-LF-B significantly suppressed the growth of B16-BL6 cells throughout the examination period, whereas Lfcin-B showed inhibitory activity only during the early period (8 days). In spontaneous metastasis of B16-BL6 melanoma cells, multiple administration of both apo-LF-B and Lfcin-B into tumor-bearing mice significantly inhibited lung metastasis produced by B16-BL6 cells, though only apo-LF-B exhibited an inhibitory effect on tumor growth at the time of primary tumor amputation (on day 21) after tumor inoculation. These results suggest that apo-LF-B and Lfcin-B inhibit tumor metastasis through different mechanisms, and that the inhibitory activity of LF-B on tumor metastasis may be related to iron (Fe^{3+})-saturation.

Key words: Bovine lactoferrin — Bovine lactoferricin — Host defense — Tumor metastasis — Antitumor activity

Lactoferrin (LF), an iron-binding glycoprotein with a molecular weight of about 80,000, is found in most biological fluids of mammals[1,2] and is released from neutrophil granules during inflammatory responses.[3] Human milk is particularly rich in LF, which is present at about 1 and 7 g/liter in natural milk and colostrum, respectively.[4] Bovine milk also contains LF at the concentration of 0.1 g/liter in mid-lactation.[4] Various biological functions of human LF (LF-H) and bovine LF (LF-B) have been demonstrated in host defense, especially in immune responses,[5,6] antibacterial activity[7,8] and transcriptional activation of cells.[9] As regards immunological functions of LF, it has been shown that LF activates natural killer (NK) cells,[10,11] polymorphonuclear leukocytes (PMN)[12] and lymphokine-activated killer (LAK) cells,[10] induces colony-stimulating activity,[13] and enhances antibody-dependent cellular cytotoxicity (ADCC)[14] and macrophage cytotoxicity.[12] Thus, a wide array of biological functions of LF is considered to be mainly related to host primary defense mechanisms. Despite the diverse functions attributed to LF, the spectrum and role of its activities in primary host defense have not yet been fully elucidated. Furthermore, the iron-binding function of LF appears not to be related to a number of these activities.[14,15]

Bovine lactoferricin (Lfcin-B), an LF-B-derived peptide consisting of 25 amino acid residues generated by acid-pepsin hydrolysis,[16] was shown to have strong bactericidal activity against a wide range of microorganisms.[17,18] The antimicrobial activity of this peptide was demonstrated to be much greater than that of LF-B, so that Lfcin-B is considered to be the active domain responsible for antimicrobial activity of LF-B. Even though many investigators have examined the biological activities of LF and LF-derived peptides, there is little direct evidence that these molecules have antitumor activities. Recently, Bezault *et al.*[19] demonstrated that LF-H suppressed the growth of tumor cells, MCA4-P5 fibrosarcoma and v-*ras*-transformed NIH 3T3, and inhibited experimental metastasis of B16-F10 melanoma cells in

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mice, and they suggested that the antitumor activity of LF-H might be mediated by NK cells and be independent of iron-saturation level. However, the biological activity against tumors of LF-B, which is 69% identical to LF-H over the entire amino acid sequence[20] and possesses similar physiological functions to LF-H, is better understood.

In the present study, we investigated the effects of LF-B and Lfcin-B on tumor metastasis using established tumor models in mice, and compared the activities of LF-B and LF-H. We found that iron-free LF-B (apo-LF-B) and Lfcin-B, but not iron-bound LF-Bs (holo-LF-B and native-LF-B), inhibited experimental tumor metastasis (lung and liver colonization), whereas apo-LF-H did not exhibit any activity in our experimental models. These results may be relevant to the mechanism of the antimetastatic effect attributed to LF-B and Lfcin-B, and indicate a potential function of this multifunctional milk protein and its peptide in primary host defense against tumor development and progression.

MATERIALS AND METHODS

Animals Specific pathogen-free female C57BL/6 and CDF_1 mice, 7–8 weeks old, were purchased from Shizuoka Laboratory Animal Center, Hamamatsu. Mice were maintained in the Laboratory of Animal Experiments, the Institute of Immunological Science, Hokkaido University, under laminar air-flow conditions. Water and pelleted diets (Nihon Nosan Kogyo, Co., Ltd., Yokohama) were supplied *ad libitum.*

Cell lines and cell cultures A lung metastatic subline of murine B16 melanoma, B16-BL6 cells,[21] was maintained as a monolayer culture in Eagle's MEM supplemented with 5% fetal bovine serum (FBS), vitamin solution, sodium pyruvate, non-essential amino acids and L-glutamine. Liver metastatic L5178Y-ML25 T-lymphoma cells[21] (partially metastasizing to the spleen) were maintained in RPMI-1640 supplemented with 5% FBS and L-glutamine.

Reagents LF-B was isolated from bovine milk whey according to the reported method.[22] LF-H was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO). Apo-LF and iron-saturated LF were prepared as described previously.[23] Briefly, apo-LF was prepared as follows: LF solution was dialyzed against 0.1 *M* citric acid for 24 h, then against pure water for 48 h, and lyophilized. For iron (Fe^{3+})-saturated lactoferrin preparation, LF solution was dialyzed against 0.05 *M* sodium bicarbonate-citric acid (pH 7.5) containing 10 mg/liter of iron (III) ammonium citrate. Thereafter, LF solution was dialyzed against pure water for 48 h for removal of unbound iron, and the iron-saturated LF was lyophilized. The lyophilized LFs were stored at $-20°C$ and dissolved in phosphate-buffered saline (PBS, 0.01 *M*, pH 7.0) before use. Holo-LF and native-LF mean 100%- and 30%- saturated LF, respectively. Purified Lfcin-B (FKCRRWQWRMKKLGAPSITCVRRAF), a cationic peptide corresponding to residues 17–41 near the N-terminus of LF-B,[16] was kindly donated by the Nutritional Science Laboratory, Morinaga Milk Industry Co., Ltd.

Experimental lung metastasis or liver and spleen metastasis Experimental lung metastasis of B16-BL6 cells was assessed by i.v. inoculation of B16-BL6 cells into C57BL/6 mice as described previously.[21] In the lung metastasis experiment, mice were given i.v. administration of various LFs (1 mg/mouse) or Lfcin-B (0.5 mg/mouse) 1 day after i.v. inoculation of 4×10^4 B16-BL6 melanoma cells. The mice were killed 14 days after tumor inoculation and their lungs were fixed in Bouin's solution. Lung tumor colonies were counted under a dissecting microscope. The assay for liver and spleen metastasis was conducted by i.v. inoculation of 4×10^4 L5178Y-ML25 cells into CDF_1 mice as described previously.[24] The weights of the liver and spleen were recorded 14 days after tumor inoculation to evaluate tumor metastasis.

Spontaneous lung metastasis C57BL/6 mice were inoculated s.c. with 5×10^5 B16-BL6 cells into the right footpads and the primary tumors were surgically removed 21 days after tumor inoculation.[21] The tumor-bearing mice received i.v. administration of LF-B (1 mg/mouse) or Lfcin-B (0.5 mg/mouse) three times at 3-day intervals before or after tumor amputation. The mice were killed 35 days after tumor inoculation and lung tumor colonies were counted under a dissecting microscope.

Inhibition assay of tumor growth *in vivo* Three C57BL/6 mice per group were inoculated s.c. with B16-BL6 (4×10^5/site) melanoma cells into the right and left footpads. The mice were treated s.c. with various LFs (1 mg/mouse) or Lfcin-B (0.5 mg/mouse) 1 day after tumor inoculation. The diameter of the tumor mass was measured from day 8 after tumor inoculation.

Assay of tumor-induced angiogenesis The *in vivo* assay of tumor angiogenesis was carried out as described previously.[25] C57BL/6 mice were inoculated i.d. with B16-BL6 melanoma cells (5×10^5) at two sites on the back and treated s.c. with various LFs (1 mg/mouse) or Lfcin-B (0.5 mg/mouse) 1 day after tumor inoculation. Eight days after tumor inoculation, mice were killed immediately after i.v. injection (0.2 ml/mouse) of 1% Evan's blue solution, and the skins were separated from the underlying tissues. Each of the inoculation sites was located under a dissecting microscope, and angiogenesis was quantitated by counting the number of vessels oriented toward the tumor mass. At the same time, the tumor size was assessed by averaging the diameter of the short and long axes of the remainder of the injected cells.

All counts were made by a single observer in a blinded manner.

Assay of NK cell activity Two C57BL/6 mice were treated s.c. with 1 mg/mouse of various LFs or 0.5 mg/mouse of Lfcin-B, and splenocytes were harvested 1, 2, 3, 4 and 5 days after administration. Single cell suspensions (100 μl/well) of splenocytes were added to [^{125}I]-IdURd-labeled Yac-1 cells (1×10^4/100 μl/well) to obtain an effector cells (splenocytes): target cell (Yac-1) ratio (E/T ratio) of 100 : 1, 50 : 1 or 25 : 1 in a round-bottomed 96-well plate, and the cultures were incubated for 6 h at 37°C in a 5% CO_2-air atmosphere. After incubation, the plate was centrifuged for 10 min at 900*g*. The supernatant (100 μl) from each well was absorbed on cotton swabs and monitored for radioactivity using a gamma counter. The percentage cytotoxicity generated by NK cells was calculated from the radioactivity (count min^{-1}) according to the following formula:

$$\text{Cytotoxicity (\%)} = [(\text{experimental release} - \text{spontaneous release})/(\text{maximum release} - \text{spontaneous release})] \times 100$$

Statistical analysis The statistical significance of differences between groups was calculated by applying Student's two-tailed *t* test.

RESULTS

Effect of LF-B and Lfcin-B on liver and spleen metastasis of lymphoma cells The antimetastatic effect of various LFs, such as apo-LF-H, apo-LF-B and holo-LF-B, and Lfcin-B was assessed on liver and spleen metastasis of L5178Y-ML25 tumor cells. Table I shows that s.c. administration of apo-LF-B (1 mg/mouse) as well as Lfcin-B (0.5 mg/mouse) 1 day after tumor inoculation significantly inhibited liver and spleen metastasis produced by L5178Y-ML25 lymphoma cells. However, neither apo-LF-H nor holo-LF-B at the dose of 1 mg/mouse showed any inhibitory effect on liver and spleen metastasis. In addition, native-LF-B (1 mg/mouse) did not inhibit tumor metastasis of L5178Y-ML25 cells (data not shown).

Effect of LF-B and Lfcin-B on lung metastasis of tumor cells We next examined the effect of LF-B and Lfcin-B on inhibition of lung metastasis produced by B16-BL6 melanoma cells. As described in Table II, s.c. administration of apo-LF-B and Lfcin-B 1 day after tumor inoculation had a therapeutic effect on lung metastasis of tumor cells. In addition, apo-LF-B administered i.v. and intraperitoneally (i.p.) showed the same activity to inhibit lung metastasis of tumor cells. However, in accordance

Table II. Inhibitory Effect of LF-B and Lfcin-B on Lung Metastasis Produced by i.v. Inoculation of B16-BL6 Melanoma Cells

Treatment	Dose (mg/mouse)	Route	Number of lung metastases: Mean±SD (% inhibition)	Range
Exp. 1				
Untreated (tumor control)			125±25	(102–129)
apo-LF-H	1	s.c.	118±7	(112–127)
apo-LF-B	1	s.c.	60±10 (47.8)*	(48–70)
holo-LF-B	1	s.c.	108±35	(72–155)
Lfcin-B	0.5	s.c.	64±4 (44.3)*	(60–68)
Exp. 2				
Untreated (tumor control)			93±19	(80–123)
apo-LF-B	1	i.v.	49±6 (47.3)*	(42–53)
	1	i.p.	50±12 (46.2)*	(36–66)

Five C57BL/6 mice per group were inoculated i.v. with 4×10^4 B16-BL6 melanoma cells and treated s.c., i.v. or i.p. with the indicated doses of various LFs or Lfcin-B 1 day after tumor inoculation. Mice were killed 14 days after tumor inoculation.
* $P<0.001$, compared with the untreated group by Student's two-tailed *t* test.

Table I. Inhibitory Effect of LF-B and Lfcin-B on Liver and Spleen Metastasis Produced by i.v.-Inoculation of L5178Y-ML25 Lymphoma Cells

Treatment	Dose (mg/mouse)	Mean weight (g)±SD (% inhibition): Liver	Spleen
Untreated (tumor control)		2.86±0.7	0.20±0.04
apo-LF-H	1	2.84±0.6	0.22±0.01
apo-LF-B	1	1.44±0.3 (49.7)*	0.13±0.03 (35)*
holo-LF-B	1	2.65±1.2	0.19±0.04
Lfcin-B	0.5	1.53±0.5 (46.5)*	0.15±0.03 (25)*
(Normal mice)		1.00±0.1	0.08±0.02

Five CDF_1 mice per group were inoculated i.v. with 4×10^4 L5178Y-ML25 lymphoma cells and treated s.c. with the indicated doses of various LFs or Lfcin-B 1 day after tumor inoculation. Mice were killed 14 days after tumor inoculation.
* $P<0.05$, compared with the untreated group by Student's two-tailed *t* test.

with the results of Table I, apo-LF-H and holo-LF-B did not have any inhibitory effect on tumor metastasis. Since the antitumor activity of LF-H is mediated by enhancement of NK activity,[19] we examined whether the antimetastatic activity of apo-LF-B and Lfcin-B was also related to activation of NK cells. However, we failed to obtain data to support the results in Tables I and II; the extents of lysis (%) of NK-sensitive cells (YAC-1) were 15±2.2, 36±4.8, 27±2.6, 25±3.2 and 27±1.3 for the non-treated control, and the apo-LF-H-, apo-LF-B-, holo-LF-B- and Lfcin-B-treated groups, respectively, on day 2 after treatment, this being the time when all groups exhibited maximal activity during the period of 5 days after treatment.

Table III. Inhibitory Effect of LF-B and Lfcin-B on Tumor Growth and Tumor-induced Angiogenesis

Treatment	Dose (mg/mouse)	Tumor size (mm) (mean±SD)	No. of blood vessels (mean±SD)
Untreated (tumor control)		8.5±0.7	13±2.6
apo-LF-H	1	8.1±0.6	10±2.8
apo-LF-B	1	7.3±1.0*	8±1.5**
holo-LF-B	1	8.3±0.5	14±4.6
Lfcin-B	0.5	7.5±0.6*	7±1.4***

Three C57BL/6 mice per group were inoculated i.d. with 5×10⁵/site of B16-BL6 melanoma cells at two sites on the back and treated s.c. with the indicated doses of bovine lactoferrins or lactoferricin 1 day after tumor inoculation. Mice were killed 8 days after tumor inoculation for evaluation.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$, compared with the untreated group by Student's two-tailed *t* test.

Inhibitory effect of LF-B and Lfcin-B on tumor-induced angiogenesis We next investigated the inhibitory effect of LF-B and Lfcin-B on tumor-induced angiogenesis. Table III shows that s.c. administration of apo-LF-B and Lfcin-B, but not apo-LF-H and holo-LF-B, 1 day after tumor inoculation resulted in a significant inhibition of tumor-induced angiogenesis and suppression of tumor growth on day 8 after tumor inoculation. These results indicate that apo-LF-B and Lfcin-B inhibit tumor metastasis and their antimetastatic effect is related to the inhibition of tumor-induced angiogenesis.

Effect of LF-B and Lfcin-B on tumor growth *in vivo* The results in Table III indicate that apo-LF-B and Lfcin-B suppress tumor growth on day 8 after tumor inoculation. To examine their activity to suppress tumor growth in detail, we carried out a long-term analysis of tumor growth inhibition of various LFs and Lfcin-B for up to 21 days after tumor inoculation *in vivo*. As shown in Fig. 1, single administration of apo-LF-B, but not holo-LF-B or LF-H, significantly inhibited tumor growth throughout the examination period. However, the administration of Lfcin-B showed just a temporary inhibition of tumor growth until 8 days after tumor inoculation.

Inhibition of spontaneous lung metastasis by LF-B and Lfcin-B Tumor-bearing mice were administered i.v. with apo-LF-B or Lfcin-B 3 times in total at 3-day intervals before or after the excision of primary tumors on day 21. The administration of apo-LF-B (1 mg/mouse) 1, 4 and 7 days after tumor inoculation significantly inhibited primary tumor growth and lung metastasis of B16-BL6 melanoma cells (Table IV). In addition, apo-LF-B treatment after the excision of primary tumors (on days 22, 25 and 28) resulted in a significant inhibition of lung

Table IV. Inhibitory Effect of apo-LF-B and Lfcin-B on Lung Metastasis Produced by B16-BL6 Cells in Spontaneous Metastasis Model

Treatment after tumor inoculation (on day)	Dose (mg/mouse)	Primary tumor size (mean±SD, mm)	No. of lung metastases	
			Mean±SD (% inhibition)	Range
Untreated (tumor control)		10.6±0.3	41±22	(20–74)
apo-LF-B				
1, 4, 7	1	9.0±0.8***	13±7 (68.3)*	(5–21)
22, 25, 28	1	10.2±0.2	9±6 (78.1)**	(0–17)
	0.3	10.4±0.8	9±9 (78.1)*	(0–22)
Lfcin-B				
1, 4, 7	0.5	10.4±0.5	9±4 (78.1)**	(5–14)
22, 25, 28	0.5	10.4±0.6	16±5 (60.9)*	(11–22)

Seven C57BL/6 mice per group were given s.c. the indicated doses of apo-LF-B or Lfcin-B on the indicated days after the footpad injection of B16-BL6 melanoma cells (5×10⁵). Primary tumors were surgically amputated 21 days after tumor inoculation. Mice were killed 14 days after amputation for evaluation.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$, compared with the untreated group by Student's two-tailed *t* test.

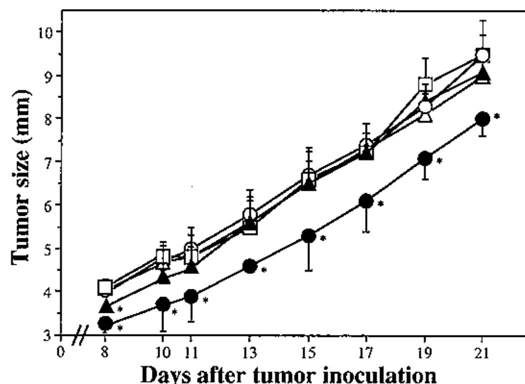

Fig. 1. Effect of LFs and Lfcin-B on the growth of B16-BL6 melanoma cells *in vivo*. Three C57BL/6 mice per group were inoculated s.c. with B16-BL6 melanoma cells (4×10^5/site) on the right and left footpads. The mice were treated s.c. with LFs (1 mg/mouse) or Lfcin-B (0.5 mg/mouse) the day after tumor inoculation. Tumor size was measured for up to 21 days after tumor inoculation. ○, the untreated control; △, apo-LF-H; ▲, Lfcin-B; □, holo-LF-B; ●, apo-LF-B. * $P<0.01$, compared with the untreated (control) group by Student's two-tailed *t* test.

metastasis, and its antimetastatic effect was apparent at the dose of 0.3 mg/mouse. Furthermore, the multiple administration of Lfcin-B caused a significant inhibition of lung metastasis in both administration schedules, but the multiple administration of Lfcin-B before the excision of the primary tumors failed to suppress tumor growth, supporting the result in Fig. 1. Thus, both apo-LF-B and Lfcin-B are able to inhibit spontaneous lung metastasis, but through different mechanisms.

DISCUSSION

Breast milk contains various components that play an important role in the defense systems of breast-fed infants against infections, in addition to providing nutrients. Among milk proteins, LF, the most avid iron-binding member of the transferrin family of proteins, is considered to be involved in primary defense against microbial infections through binding of iron required for microbial growth.[7] Lfcin-B, an LF-B-derived peptide generated by acid-pepsin hydrolysis, was also shown to have a potential antimicrobial role.[17] However, many other functions, including a much broader role in host resistance to microorganisms, have been attributed to LF and its peptides. Here we have shown that LF-B and Lfcin-B inhibit tumor metastasis and suppress tumor-induced angiogenesis in mice, and that the antitumor activity of LF-B is related to its iron-saturation.

The antitumor activity of LF-H was shown to be mediated by NK cells,[10,19] a well-known antitumor effector, and so we addressed the possibility that the discrepancy of antitumor effect between human and bovine LF resulted from a difference in their ability to enhance NK activity. Unexpectedly, every LF, including apo-LF-H, apo-LF-B and holo-LF-B, enhanced NK activity to almost the same extent, so that NK activity was not thought to be the main factor determining the difference in antitumor activity between LF-B and LF-H, even though mediation of NK activity might be related in part to the antitumor effect of apo-LF-B and Lfcin-B. This observation suggested that the antitumor activity of LF-B is mediated by a different mechanism from that of LF-H.

The multifunctional activities of LF are generally recognized to be independent of its iron-binding ability,[14,15] and Bezault *et al.*[19] reported that LF-H inhibited experimental metastasis of murine tumor cells regardless of its level of iron-saturation. We demonstrated that LF-H inhibited lung metastasis of B16-F10 melanoma cells, and its antimetastatic effect was supported by its suppressive activity on the growth of MCA4-P5 fibrosarcoma and v-*ras*-transformed NIH 3T3 cells *in vivo*. However, in our *in vivo* models, LF-H did not inhibit lung metastasis of B16-BL6 melanoma cells or liver and spleen metastasis of L5178Y-ML25 lymphoma cells, and did not suppress the growth of B16-BL6 cells, the same cell line used in the lung metastasis experiments. On the other hand, LF-B exhibited antitumor activity that was dependent on its level of iron-saturation; that is, iron-free LF-B (apo-LF-B), but not iron-bound LF-Bs (holo-LF-B and native-LF-B), inhibited tumor metastasis and suppressed tumor growth. Furthermore, apo-LF-B, but not apo-LF-H, inhibited tumor-induced angiogenesis, an activity which was considered to be related to the suppression of tumor growth. Therefore, the antitumor activity of apo-LF-H seems to be dependent on the tumor systems and cell lines used for metastasis experiments.

Considering that iron is required for tumor cell proliferation and iron chelation reduces tumor cell growth,[26] it is possible that the inhibitory effects of apo-LF-B on tumor growth resulted from iron chelation, since iron-free LF is able to bind much more iron than iron-saturated LF. However, the reason why apo-LF-H, the same iron-free protein as apo-LF-B, could not inhibit tumor metastasis remains unclear. Apo-LF-B and apo-LF-H may possess distinct physiological properties, such as strength of iron binding or chelation, and this may account in part for the discrepancy in antitumor activity between these apo-LFs, in addition to the difference in tumor systems used.

Lfcin-B is a cationic peptide comprising the active domain of LF-B for its antimicrobial activity.[16-18,27] The antibacterial spectrum of Lfcin-B has been extensively

studied, though other biological functions of Lfcin-B have not yet been examined. We have shown here for the first time that Lfcin-B inhibits tumor metastasis and angiogenesis. Lfcin-B showed lower activity than apo-LF-B; Lfcin-B inhibited tumor growth only at the early period (Table III), whereas apo-LF-B maintained long-term activity (Fig. 1). Since Lfcin-B lacks both tyrosine and histidine residues which are essential for the iron-chelating functions of LF-B,[28] the mechanism of antitumor activity of this peptide should not involve an iron-chelating or iron-binding function. Many other peptides with similar cationic features to Lfcin-B exert their lethal effect against microorganisms by disrupting cell membrane functions.[28, 29] Thus, the antitumor activity of Lfcin-B probably involves such a mechanism.

The present study has demonstrated that apo-LF-B and Lfcin-B inhibit experimental and spontaneous metastasis of hematogenous and non-hematogenous tumor cells in mice, and that the antitumor activity of LF-B is related to its iron-binding ability. Further studies are in progress to elucidate more fully the mechanism of the effects of apo-LF-B and Lfcin-B on tumorigenesis, and to examine the potential for application of these compounds as therapeutic agents.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Mamoru Tomita, Food Research and Development, Morinaga Milk Industry Co., Ltd., Japan, for his kind gift of purified Lfcin-B, and Ms. S. Kuriyama and M. Sato for their skillful technical assistance. This work was supported in part by the Oasaka Foundation for Promotion of Clinical Immunology and by Grants-in-Aid for Special Project Research from Hokkaido University provided by the Ministry of Education, Science, Sports and Culture.

(Received October 7, 1996/Accepted December 3, 1996)

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