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Abstract

The effects of surgical intervention by removal of the primary focus, and the effectiveness of an immunomodulator, Corynebacterium parvum (Cp), on the proliferation of metastatic tumor tissue were investigated by following the postoperative changes in the 3H-thymidine labelling rate of metastatic tissue in an experimental model of metastasis in mice. In addition, the delayed type hypersensitivity reaction (DTH) was studied to investigate the immune capacity of the host. The labelling rate of mice that had the primary focus removed remained high with little variation, while that of the mice not operated on decreased gradually. On the other hand, in mice undergoing a sham operation, the rate was the same as that of the mice with the primary focus removed for a short while, but then gradually decreased. When Cp was administered, especially before removal of the primary focus, the rate was lower than that of the tumor bearing control group and decreased steadily. The number of pulmonary metastatic nodules was increased by removal of the primary focus, but this increase was inhibited by the administration of Cp which prolonged life. The depression in the DTH was less in the group given Cp preoperativeLy than in either the group of mice having the primary focus removed or those not having it removed.

KEYWORDS: metastatic tumor, surgical intervension, autoradiography, Corynebacterium parvum

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EFFECTS OF THE REMOVAL OF THE PRIMARY TUMOR AND IMMUNOTHERAPY WITH *CORYNEBACTERIUM PARVUM* ON METASTATIC TUMOR PROLIFERATION

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Abstract. The effects of surgical intervetion by removal of the primary focus, and the effectiveness of an immunomodulator, *Corynebacterium parvum* (Cp), on the proliferation of metastatic tumor tissue were investigated by following the postoperative changes in the ^{3}H-thymidine labelling rate of metastatic tissue in an experimental model of metastasis in mice. In addition, the delayed type hypersensitivity reaction (DTH) was studied to investigate the immune capacity of the host. The labelling rate of mice that had the primary focus removed remained high with little variation, while that of the mice not operated on decreased gradually. On the other hand, in mice undergoing a sham operation, the rate was the same as that of the mice with the primary focus removed for a short while, but then gradually decreased. When Cp was administered, especially before removal of the primary focus, the rate was lower than that of the tumor bearing control group and decreased steadily. The number of pulmonary metastatic nodules was increased by removal of the primary focus, but this increase was inhibited by the administration of Cp which prolonged life. The depression in the DTH was less in the group given Cp preoperatively than in either the group of mice having the primary focus removed or those not having it removed.

Key words : metastatic tumor, surgical intervention, autoradiography, *Corynebacterium parvum.*

It is commmonly experienced, both clinically and in animal experiments, that tumor tissue remaining in the cancer-bearing host goes on to proliferate after an operation to remove the tumor. Various possibilities have been put forward to explain this phenomenon, one of which considers changes in the immune capacity of the host important (1). In the present experiments, the role of surgical intervention and the effectiveness of immunological activators against the proliferation of metastatic tumor tissue was investigated by following the postoperative changes in the ^{3}H-thymidine labelling rate of metastatic tumor tissue in an experimental model of meatstasis in mice. In addition, the effectiveness of *Corynebacterium parvum* (Cp) (2) was studied in conjunction with the delyaed type hypersensitivity reaction (DTH).

MATERIALS AND METHODS

The primary focus was an inoculation of 5×10^6 Lewis lung cancer cells made up as a single cell suspension by treatment with trypsin. The inoculation was given subcutaneously into the right hindleg of BDF_1 female mice aged between 8 and 10 weeks. The mice were then divided into the following groups : 1. mice in which the primary focus was not removed, 2. those in which the primary focus was removed, 3. those undergoing a sham operation, 4. those to which Cp was administered before removal of the primary focus, and, 5. those to which Cp was administered after removal of the primary focus.

Removal of the primary focus. On the 14th day after the tumor cell inoculation, the right hindleg (groups 2, 4 and 5) or the left hindleg (group 3) was amputated at the pelvis under ether anesthesia.

Cp administration. Cp was given into the abdominal cavity daily for 5 days beginning on the 9th day in group 4 and on the 15th day in group 5. The dose was 0.4 mg/mouse/day.

Autoradiography. ^{3}H-thymidine was given intra-peritoneally at a dose of 10 μCi/kg of body weight. One hour later, the mice were sacrificed by decapitation. The lungs were removed en bloc, fixed in 10 % buffered formalin, embedded in paraffin, and sequential sections made. The sections were dipped in Sakura emulsified agent $NR\text{-}M_2$ and developed after 4 weeks exposure at 4 ℃. H-E staining was performed, and a total of more than 3,000 tumor cells was counted in 3 different places for each specimen, following the maximum diameter of the lung metastatic focus. The labelling rate was then cultulated.

Number of macroscopic lung metastatic nodules. The number of nodules was estimated on the 24th day using the intratracheal india ink infusion method of Wexler (3).

DTH. In addition to the 5 groups above, a normal control group and a group in which normal mice underwent amputation of the right hindleg were added. A 6 % solution of picril chloride in ethanol was painted on the abdominal skin at the time of tumor inocuration, and on the 9th and 18th days to cause sensitization. In each case, a 1 % solution of picril chloride in olive oil was painted on both ears seven days later. Twenty-four hours later, the thickness of each ear was measured with a dial thickness gauge. The increase in the thickness of the ear was calculated by subtracting the average background value from the average for the difference in thickness before and after application of the paint.

RESULTS

The effect of surgical intervention on the proliferation of metastatic tumors. Comparison of the labelling rate in the mice that had the primary focus removed with that of those that did not showed that the former had a higher value of around 50 % with little variation. In contrast to this, the non-removal group had a value of 50.5 % on the 16th day which because significantly low on the 18th day and decreased steadily thereafter to be 32.6 % on the 28th day. On the other hand, in the sham operation group, the rate was not different from that of the removal group up to the 18th day, but was significantly lower from the 21st day onwards (Fig. 1).

The inhibitory effect of Cp on proliferation of remnant metastatic tumor. When Cp was administered before the operation, the rate was significantly lower on the 16th day decreased steadily so that on the 28th day it was 27.6 %, indicating a marked inhibition of metastasis. However, a value of 36 % on the 31st day

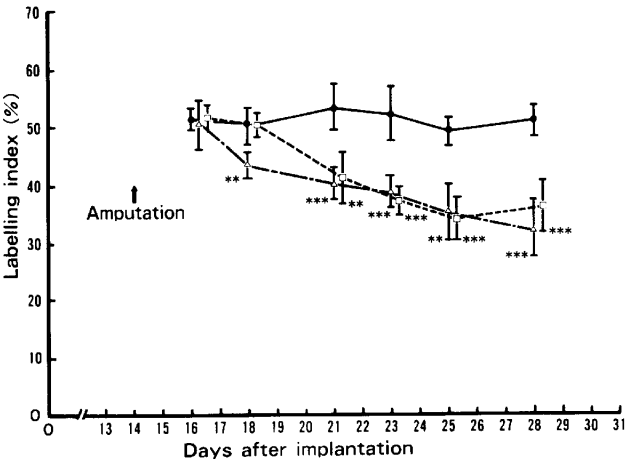

Fig. 1. Labelling indexes of metastatic Lewis lung carcinoma. (●-●) amputated (control), (△-△) nonamptated, (□-□) sham operation, ** : $p<0.01$, *** : $p<0.001$.

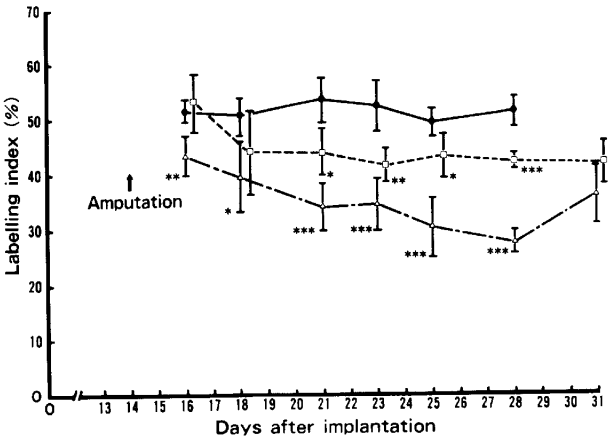

Fig. 2. Labelling indexes of metastatic Lewis lung carcinoma in mice administered *Corynebacterium parvum*. (●-●) : amputated (control), (△-△) : C. parvum before amputation, (□-□) : C. parvum after amputation, * : $p<0.05$, ** : $p<0.01$, *** : $p<0,001$.

indicated a weakening of the Cp effect. When Cp was administered postoperatively there was no difference up to the 18th day, but the rate became significantly low from the 21st day. However, this value was higher than that for the preoperative administration and showed no tendency to decrease (Fig. 2).

The effects of Cp and surgical intervention on the DTH. The DTH of the cancer-bearing mice was not different from that of the control group, but the DTH value tended to decrease with the advance of the cancer. On the 16th day after the inoculation of thc cancer, the DTH decreased significantly in all groups other

than the normal control, and even the group that had the primary focus removed showed no improvement. However, the DTH did not decrease in the group of normal mice that underwent amqutation of the hindleg. Comparison of groups othar than the normal control group showed that the group given Cp preoperatively had a significantly higher value and less of a decrease in DTH than the group with the primary focus removed. This effect was evident even on the 25th day (Table 1).

The effects of Cp and surgical intervention on the number of pulmonaly metatsatic nodules. The average number of lung metastatic nodules in each group was : non-removal group, 8.7 ; removal group, 36.0 ; sham operation group, 14.4 ; Cp preoperative administration group, 6.3 ; and Cp postoperative administration group, 8.5. Removal of the primary focus caused a significant ($p<0.001$) increase in the number of metastatic nodules, but this was significantly ($p<0.001$) inhibited by the preoperative adminirtration of Cp.

Survival time. The average survival time in days for each group was : non-removal group, 31.6 ; removal group, 30.0 ; sham operation group, 26.2 ; preoperative Cp group, 37.5, and postoperative Cp, 34.4. Preoperative Cp administration gave significantly ($P<0.001$) prolongation of life.

TABLE 1. EFFECTS OF SURGERY AND *CORYNEBACTERIUM PARVUM* ON THE INCREASE IN EAR THICKNESS

Groups	Days after implantation		
	7	16	25
Healthy control	2.17± 0.62 100% (9)	3.62± 0.97 100% (9)	3.83± 1.45 100% (9)
Healthy+Surgery[a]		3.75± 1.12 ○○○ 103.6% (10)	2.95± 1.50 ○○ 77.0% (10)
Nonamputated (Tumor bearing)	1.90± 0.43 87.6% (10)[b]	1.26± 1.08*** 34.8% (10)	0.58± 0.44*** 15.1% (6)
Amputated control[a]		1.59± 0.82*** 43.9% (11)	1.25± 0.96*** 32.6% (11)
Sham op.[a]		2.05± 0.61*** 56.6% (10)	0.50± 0.50* 13.1% (2)
C.p. before amputation[a]		2.50± 0.64** 69.1% (11) ○○	2.45± 1.48 ○ 64.0% (10)
C.p. after amputation[a]		1.95± 1.03** 53.7% (10)	1.05± 1.21*** 27.4% (10)

Ear thickness ($\times 10^{-2}$mm) expressed as the mean ± SD. * : $p<0.02$, ** : $p<0.01$, *** : $p<0.001$ vs. healthy control group, ○ : $p<0.05$, ○○ : $p<0.02$, ○○○ : $p<0.001$ vs. amputated control group. *a* : Surgical procedures were performed on the 14th day after post-implantation. Numbers of mice are given in parentheses. *b* : Percent of healthy control group.

DISCUSSION

The reasons why metastatic foci tend to proliferate after the removal of a primary tumor are deeply related to such factors as what side the tumor is on, antigenicity, surgical intervention (4, 5) and various immunological factors including the interation between the primary and metastatic foci (6) and concomitant immunity (7). In the present experiment, the sham operation group showed greater proliferation of lung metastases over a short time than the group that did not have the primary tumor removed. This tendency was probably related to the surgical intervention ; however, it was only transitory. The presence of a primary tumor inhibited metastatic proliferation, probably because concomitant immunity was preserved thus inhibiting metastasis. Immunotherapy using nonspecific immunomodulators has been suggested as a means of inhibiting metastasis. Cp is an immunomodulator which, given systemically, activates macrophages (8); hence, when given before removal of the primary focus, it inhibits the continuing proliferation of the remnant metastatic foci, and inhibits the decrease in the immunological parameter, DTH. Olivotto (9) reported that lung macrophages from CBA mice inhibited the DNA synthesis of RI leukemia cells *in vitro* most effectively 14 days after Cp injection, and in this experiment the most suppressive effect on the labelling index of the metastatic nodules was seen on the 15th day after Cp injection. These results suggest that macrophages activated by Cp decrease the labelling index of lung nodules. However, Cp given alone was limited in its effect of inhibiting the proliferation of metastatic tumor, and perhaps would be more effective if used in combination with chemotherapy.

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