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The Facilitation of Tumour Growth in the Lung by Cyclophosphamide in Artificial and Spontaneous Metastases Models*

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Abstract—*It had been shown previously that treatment of mice with cyclophosphamide before i.v. inoculation of tumour cells enhanced the growth of lung colonies. Results presented here show that treatment with cyclophosphamide after i.v. inoculation of tumour cells could also decrease host resistance against growth of lung colonies. This enhancement could be counteracted by pretreatment with cortisone acetate and is hardly influenced by increasing the tumour load or by dose fractionation of cyclophosphamide. In addition, the increased lung colony formation as found in the artificial model, with i.v. inoculation of tumour cells, was also present in a tumour model in which spontaneous metastases occur. However, the growth promoting effect of cyclophosphamide is for most tumours quantitatively negligible in comparison to the antitumour effect of the drug.*

INTRODUCTION

THE YIELD of lung colonies after i.v. administration of tumour cells has been found to be increased in mice pretreated with cyclophosphamide [1–7]. Furthermore treatment with cyclophosphamide also increased the take of tumour cells if inoculated subcutaneously (s.c.) [3] or intramuscularly (i.m.) [6]. Although the mechanism responsible for this effect of cyclophosphamide is not yet elucidated, a large amount of information has been obtained which excludes some possible mechanisms. As mentioned in the Discussion, a number of reasons argue against the involvement of classical immunological (T-cell dependent) mechanisms. Nevertheless, for most experimental tumours, including non-immunogenic tumours, the take rate of tumour cells after i.v. or s.c. inoculation is very low, since only one lung colony or s.c. tumour is obtained from about 10^3 to 10^5 injected tumour cells. This means that the great majority of cells die. The factors which determine the death or survival of these cells are not yet clear. For that reason, in this paper the probability of tumour cells to survive and form a lung colony is assumed to be dependent on "host resistance", whatever the mechanism is. This host resistance is apparently decreased in cyclophosphamide treated mice.

This paper describes experiments in which cyclophosphamide is administered after the i.v. inoculation of tumour cells. Furthermore, it was studied whether the facilitation of lung colony growth in cyclophosphamide treated mice is also operative in some models measuring the rate of spontaneous tumour metastases formation. The increase in lung colonies in cyclophosphamide pretreated mice has been shown to be paralleled by an increase in retention of radioactively labelled tumour cells in the lung at very early time intervals after i.v. inoculation of these cells [2, 5]. Nevertheless, the possibility could not be excluded that a similar effect would also be evident if cyclophosphamide was given after tumour cell inoculation. This paper describes experiments showing that this is indeed the case; in addition, studies were performed to investigate whether this facilitation of lung colony growth is also operative in some models for enhancing spontaneous tumour metastases.

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MATERIALS AND METHODS

Mice and tumours

Studies were performed in four tumours. The mouse osteosarcoma C22LR [1] and the Lewis lung tumour [8], both transplanted in C57BL/Rij × CBA/Rij F_1 hybrid mice, have been described earlier. The osteosarcoma has been found not to be immunogenic in extensive studies in the hybrid mice, the host of origin. The Lewis lung tumour was weakly immunogenic in the F_1 hybrid host. The other two tumours are the mammary carcinoma 2661 [9] and a subline of this tumour 2661/Cy in CBA/Rij mice. The 2661 tumour is not immunogenic in its host of origin [9]. The subline 2661/Cy recurred 6 months after extensive cyclophosphamide treatment of the original 2661 tumour. Only subpassages 4–9 of the 2661/Cy tumour were used. Treatment of the original 2661 tumour or its subline 2661/Cy with cyclophosphamide resulted in a difference in growth delay of these tumours. Growth delay is calculated as the difference in time which is necessary for tumours in treated and control mice to reach 400% of their tumour volume at the start of treatment. The growth delay for both tumours after treatment with cyclophosphamide either as a single dose of 200 mg/kg or as divided doses with 4 equal fractions of 50 mg/kg on 4 subsequent days was as follows: (a) The original 2661 tumour gave a mean growth delay of 9 days after a single dose and 16 days after the fractionated doses; (b) The 2661/Cy subline gave a mean growth delay of 2 days both after the single and the fractionated doses.

These results indicate that the subline is less sensitive to cyclophosphamide than the original tumour.

Lung colony assay

With slight modifications, this was done as described earlier [1]: briefly, single cell suspensions were made by a combination of mechanical and enzymatic cell detachment [10] from minced tumour tissue. They were suspended in Hanks' balanced salt solution (BSS) supplemented with 5% calf serum. The administration of heavily irradiated cells to obtain more uniform lung colony yields was replaced by adding plasticized carbon microspheres ($15 \pm 5\,\mu m$) 3M Company Saint Paul Minnesota, 55101, U.S.A., as used by others [11, 12] for lung colony assay. Mice were sacrificed 15 days after i.v. inoculation of tumour cells. The number of macroscopic colonies on the surface of the lung was counted after fixation in a modified Bouin solution. Sacrifice of mice was performed at 15 days after tumour cell inoculation, since it was observed that the lung colony yield was similar 11–17 days after cell inoculation. The "survival" of the lung colonies is calculated by dividing the mean number of colonies in treated mice by the mean number of colonies in control mice. There is a large variation between the various experiments if the mean number of lung colonies in treated mice are considered. For that reason results are shown in which the relative difference between groups within one experiment was reproducible in at least one further experiment.

Spontaneous metastases

Metastases of the Lewis lung tumour were obtained in the lung after i.m. inoculation of 5×10^5 or 10^6 tumour cells into the hind legs of mice. To increase the number of lung metastases, 10^6 microspheres were inoculated i.v. 1–3 hr after tumour cell inoculation [13]. For the determination of the number of macroscopic metastases on the surface of the lung, mice were sacrificed 21 days after inoculation of tumour cells and their lungs were fixed in a modified Bouin solution.

Both the 2661 tumour and the 2661/Cy tumour metastasize to lymph nodes and/or lung in all mice inoculated with 2×10^5 tumour cells s.c. into the foot pad. In this model, mice were not sacrificed as in the Lewis lung model but their survival time was taken as an indication for their metastatic tumour load.

Tumour cell survival was determined as described earlier [14]. Briefly: tumour cell suspensions were prepared from flank tumours of mice which had been subjected to various doses of whole body irradiation. These cells were titrated and inoculated s.c. into isogenic recipient mice. Comparison of the number of cells needed to produce tumours in 50% of the inoculation sites in irradiated tumours vs control tumours provided information on the cell survival.

Treatment of mice

Corynebacterium parvum was administered as a suspension of formaldehyde killed organisms of strain CN6134 and was made available by Wellcome Foundation, Beckenham, England. A dose of 0.35 mg suspended in 0.5 ml was administered i.v. to mice. Cyclophosphamide and ifosfamide (previously also called iphosphamide or isophos-

phamide), Asta Werke A. G., Brackwede, W. Germany, were dissolved and diluted in saline for i.p. injection. Methotrexate (Lederle) was dissolved in distilled water and injected s.c., 5-fluorouracil (Roche) was diluted with saline and injected i.p. All cytostatic drugs were injected in a volume of 0.01 ml/gm mouse body weight. Cortisone acetate (Organon) was injected s.c. as a suspension in a dose of 2.5 mg per mouse. Since the crystals could be recovered more than 24 hr after injection, it was assumed that there was a prolonged release of the hormone. Thorax irradiation was carried out by exposing the cranial half of the body of mice to X-rays of a 300 kVp Philips Müller machine operating at 10 mA, HVL 3 mm Cu, 60 rad/min in animals which were anaesthesized with 60 mg/kg Pentobarbital. Removal of foot tumours was accomplished by amputation of the tumour bearing leg at the knee joint.

EXPERIMENTAL PLANS AND RESULTS

Host resistance

As reported earlier [15], treatment with increasing doses of cyclophosphamide or thorax irradiation 1–4 days after i.v. inoculation of osteosarcoma cells resulted in a lung colony survival curve which consisted of two components. It appeared that, after higher doses of cyclophosphamide or radiation, there was no progressive further decrease in lung colony numbers but a decrease in effect or even an increase in colony number. This curve differed significantly from the exponential survival curve for osteosarcoma cells that had been exposed in a flank tumour and assayed by the endpoint dilution method [16]. It was considered likely that the resulting biphasic survival curve was the consequence of two antagonistic effects of the treatment, namely, an effect on tumour cell survival and an effect on host resistance. In order to elucidate this mechanism, attempts were made to abolish host resistance by an assumedly noncytotoxic treatment. In our previous studies, no effect of pretreatment with prednisolone on lung colony formation had been observed [1]. We could, however, confirm the finding of earlier investigators [17–19] that cortisone acetate enhanced colony formation (see Table 1). When a dose of 2.5 mg per mouse was administered 1 day before tumour cell inoculation, the later treatment with cyclophosphamide, 1 day after tumour cell inoculation, indeed caused an exponential decrease in colony numbers over almost three decades (Fig. 1). A similar effect of cortisone pretreatment was observed in the Lewis lung tumour (Fig. 2). This seems to confirm the role of host resistance in causing the anomaly in the colony survival curve.

Table 1. Lung colony enhancement by pretreatment of the recipients with cortisone acetate

Dose	Day	Enhancement ratio*
2.5 mg/mouse	−1	38,10,7
	−2	8,9
	−3	8,10
	−7	7
100 mg/kg	−2	31
200 mg/kg	−2	45
400 mg/kg	−2	15
800 mg/kg	−2	10

*Ratio of mean number of lung colonies in pretreated mice/control mice.

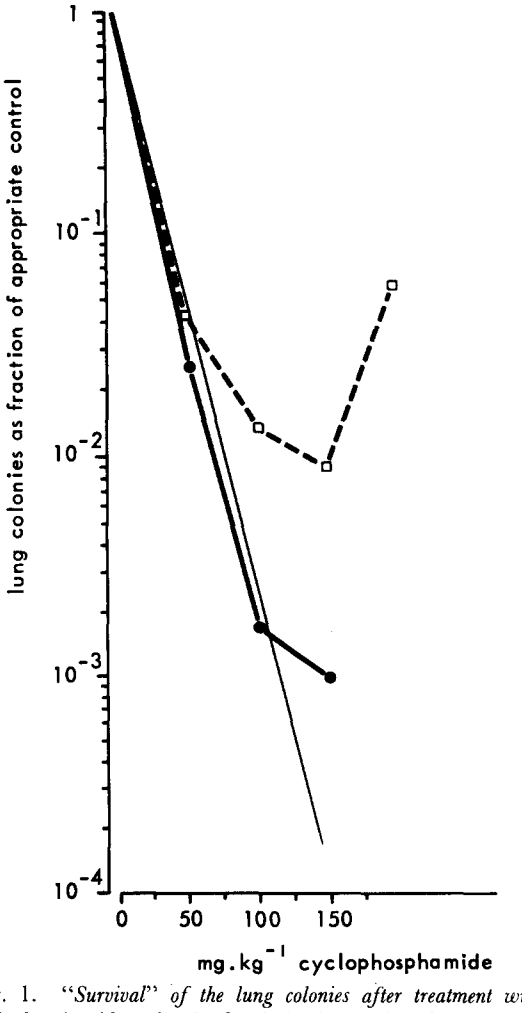

Fig. 1. "Survival" of the lung colonies after treatment with cyclophosphamide on day 1 *after the i.v. inoculation of osteosarcoma cells in mice pretreated with cortisone acetate (●——●) or control mice (□- - -□). The "survival" is determined as fraction of control mice, which received the same pretreatment but no posttreatment with cyclophosphamide. For comparison the osteosarcoma cell survival of flank tumours obtained by end point dilution assay is presented (——).*

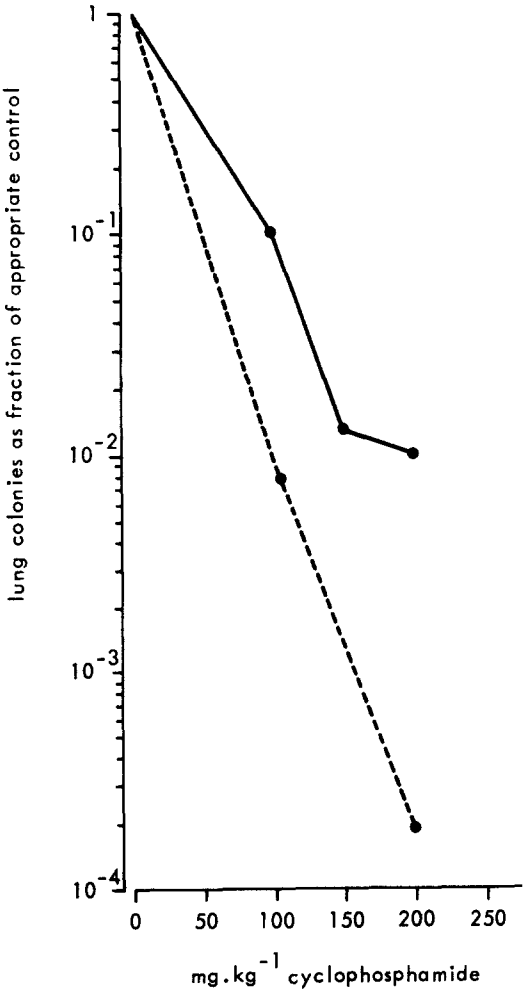

Fig. 2. "Survival" of lung colonies after treatment with cyclophosphamide on day 1 *after the i.v. inoculation of Lewis lung tumour cells in mice pretreatment with cortisone acetate (●---●) or control mice (●——●). The "survival" is determined as fraction of control mice which received the same pretreatment but no posttreatment with cyclophosphamide.*

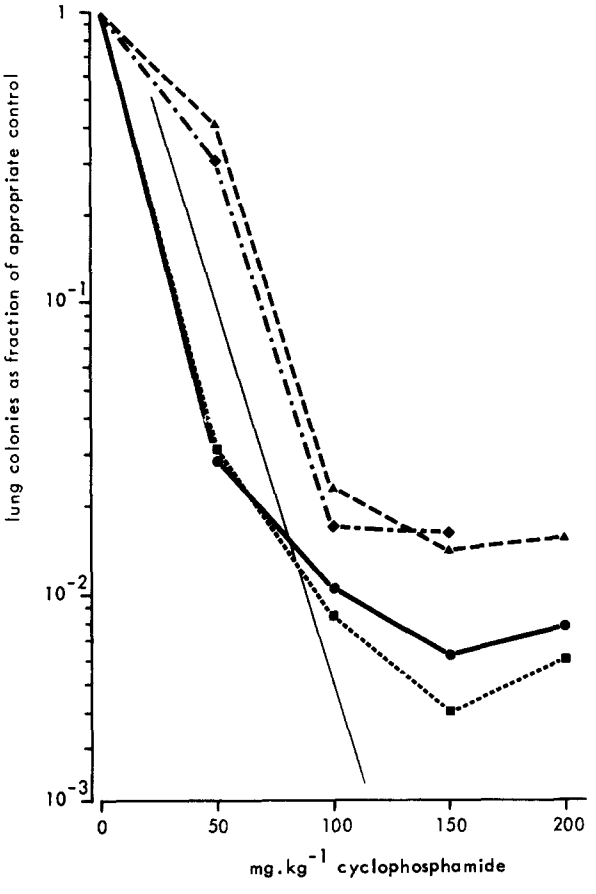

Fig. 3. Effect of fractionated administration of cyclophosphamide and of the presence of a flank tumour on the "survival" of lung colonies. The "survival" is determined as fraction of control mice, which received the same pretreatment but no posttreatment with cyclophosphamide. Mice are treated on day 1 *or on* days 1–4 *after the i.v. inoculation of osteosarcoma cells. For comparison the osteosarcoma cell survival of flank tumour obtained by endpoint dilution assay is presented: Single dose (●——●) without flank tumour; Fractionated dose (▲---▲) without flank tumour; Single dose (■---■) with flank tumour; Fractionated dose (◆---◆) with flank tumour.*

Tumour load and dose fractionation

It was next studied whether this host resistance was affected by tumour load or by fractionation of the treatment dose, two factors said to be characteristic of so-called host immunosuppression [20, 21]. For that purpose, mice were inoculated with 10^6 osteosarcoma cells s.c. 1 week before the i.v. inoculation of tumour cells. The same dose of cyclophosphamide was either administered as one single dose 1 day after tumour cell inoculation or as four equal fractionated doses on days 1, 2, 3 and 4 after tumour cell inoculation. As seen from Fig. 3, there is no effect of an additional flank tumour increasing the tumour load, nor is there a major effect of dose fractionation. The latter leads to some increase in colony survival, but this may be due to the repair of sublethal damage between fractions as expressed by a shoulder in the survival curve.

Corynebacterium parvum

In the Lewis lung tumour, it was shown that enhancement of host resistance with *Corynebacterium parvum* given 2 days before tumour cell injection increased the upward curvature of the dose–effect curve (Fig. 4) which is typical for host resistance. It should be noted that in this graph the survival in each curve is determined by comparison of the number of lung colonies in mice treated with cyclophosphamide with those in their control mice, which received the same pretreatment but no posttreatment with cyclophosphamide. If the results are presented in absolute units as colonies formed per 10^6 cells injected, it is evident that host resistance after

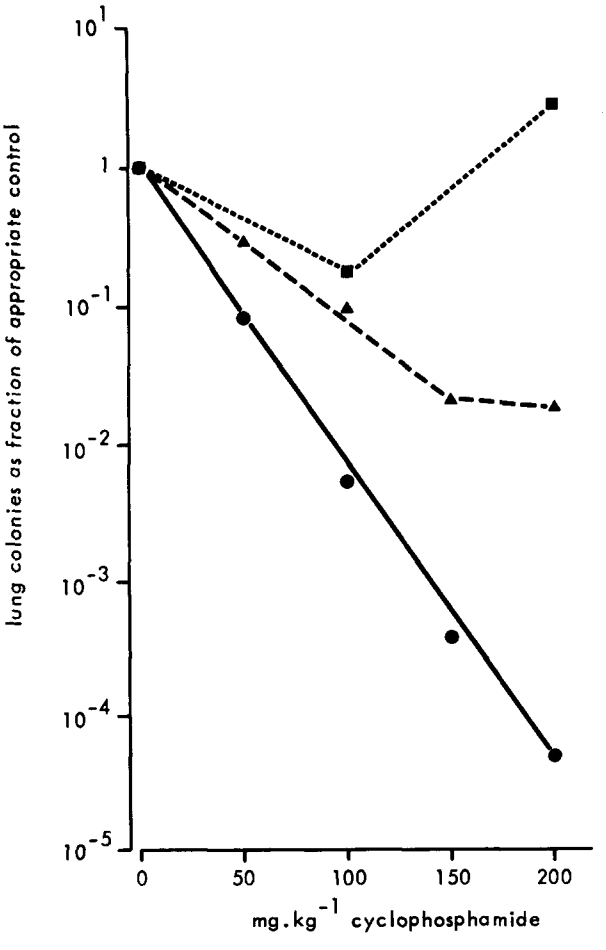

Fig. 4. "Survival" of lung colonies after treatment with cyclophosphamide on day 1 *after the i.v. inoculation of Lewis lung tumour cells in mice pretreated with cortisone acetate* (●——●). Corynebacterium parvum (■---■) *or in control mice* (▲---▲). *The "survival" is determined as fraction of control mice, which received the same pretreatment but no posttreatment with cyclophosphamide.*

150 mg/kg cyclophosphamide is similar, independent of whether the recipient was pretreated with cortisone acetate, *C. parvum* or not at all (Fig. 5).

Spontaneous metastases model

Up to now, the results have suggested that cyclophosphamide facilitates tumour formation in a variety of experimental systems. However, most of these systems are highly artificial and it was thought to be of interest to study the effect of cyclophosphamide in a system showing spontaneous metastases formation. The first study concerns Lewis lung carcinoma inoculated i.m. The effects of treatment with cyclophosphamide either 1 day before tumour inoculation, 5 days afterwards or a combination of the two on the number of lung metastases are shown in Table 2. The results indicate that the enhancement of metastases formation by pretreatment with cyclophosphamide is significant but small. The decrease in metastases number by treatment with cyclophosphamide after tumour cell inoculation is quantitatively much more important and any small effect on host resistance is unobservable in comparison with the marked effect on the tumour.

For this reason, another study was made in a tumour which was thought less sensitive to cyclophosphamide. Chemotherapy with cyclosphosphamide was applied either before or after amputation of a leg carrying tumour 2661/Cy in the foot pad. Treatment after amputation permitted a short prolongation of survival ($P<0.05$) (Fig. 6) but treatment before amputation was much more effective (Fig. 7). Evidently, the tumour was not resistant to cyclophosphamide and the antitumour effect was again more pronounced than the effect on host resistance. The better effect of treatment before amputation could be due to a smaller load of metastatic tumour or to the effect on the primary; it certainly does not show a facilitation of tumour growth through depression of host immunity.

In another series of experiments, the effect

Table 2. Effect of cyclophosphamide on the number of lung metastases seen 21 days *after i.m. inoculation of Lewis lung tumour cells on* day 0

Dose of cyclophosphamide (mg/kg)	Day of treatment	Average number of lung metastases (±S.E.)† $+10^6$ microspheres			-10^6 microspheres	
		Exp. I	Exp. II	Exp. III	Exp. II	Exp. III
0		71±9	38±7	64±13	6±2	16±3
250	−1	200	133±25*	154±27*	14±3*	22±3
150	−1		79±28*		7±2	
250	+5	1.1±0.7*	1.0±0.3*	0*	0.3±0.2*	0*
250	−1, +5		1.2±0.5*	0.7±0.6*	0.2±0.1*	0.4±0.2*

*Significant difference with control (Student's *t*-test).
†Each group consisted of at least 8 mice.

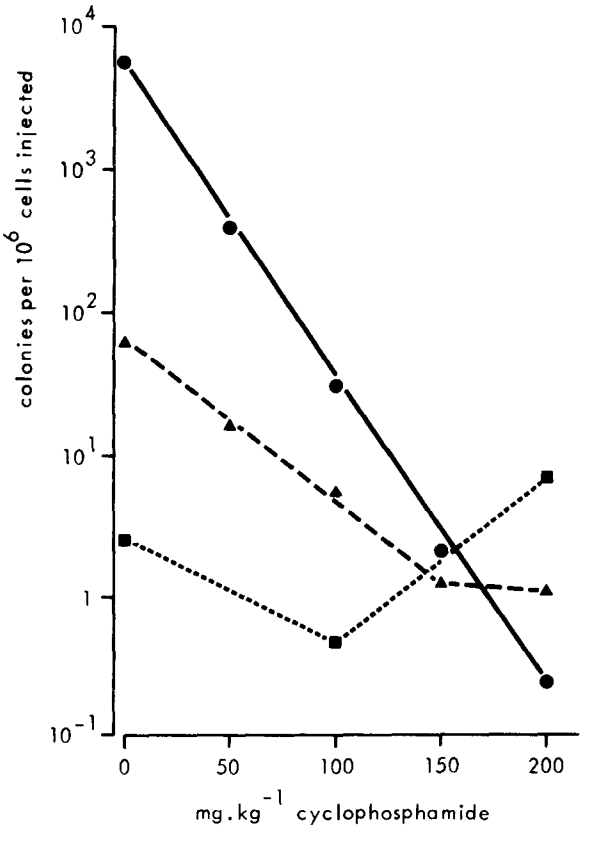

Fig. 5. These data are similar to those depicted in Fig. 4, however, the results are presented in absolute numbers of lung colonies instead of the "survival" of lung colonies compared to this appropriate control.

of adjuvant chemotherapy applied after surgical removal of a mammary tumour 2661 in the footpad was studied [9, 23, 24]. In these studies, the chemotherapy schedule which was started 3 days after surgery, was applied in 4 weekly courses of CMF (on Monday 100 mg/m^2 cyclophosphamide, 300 mg/m^2 5-fluorouracil and 20 mg/m^2 methotrexate, on Tuesday and Wednesday only 100 mg/m^2 cyclophosphamide). The results indicated that the chemotherapy treatment eliminated almost all lymph node metastases, whereas the lung metastases were not significantly decreased. In order to determine whether the low efficiency of chemotherapy with respect to the lung metastases was due to the enhancement effect of cyclophosphamide, the CMF treatment with the regimen as described before was compared with the following alternatives: MF, a treatment in which cyclophosphamide was omitted and IMF, a treatment in which cyclophosphamide was substituted for by ifosfamide at a similar dose. Ifosfamide was as equally effective as cyclophosphamide at similar dose levels in inducing growth delay of flank tumours (unpublished data), but was much less effective in abolishing host resistance [1, 2, 3]. The survival curves of mice treated with the various regimens, starting 3 days after amputation of the tumour bearing leg, are presented in Fig. 8. No significant difference is observed between the curves as determined by the log rank test [25].

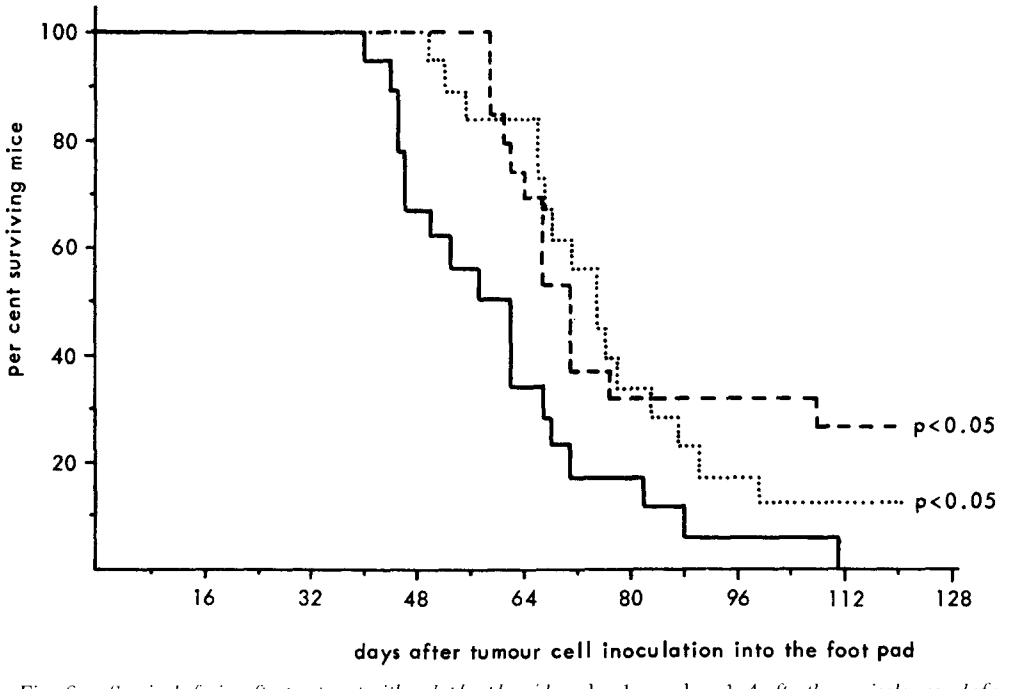

Fig. 6. Survival of mice after treatment with cyclophosphamide on day 1 *or on* days 1–4 *after the surgical removal of a* 2661/*Cy tumour growing in the foot pad. Surgery was performed* 26 days *after s.c. inoculation of tumour cells. Significance in survival was determined in comparison with the control group by log rank test. Number of surviving mice: Control* 0/18 (——); 200 mg/kg *cyclophosphamide* 5/19 (- - -); 4 × 50 mg/kg *cyclophosphamide* 2/18 (. . . . .).

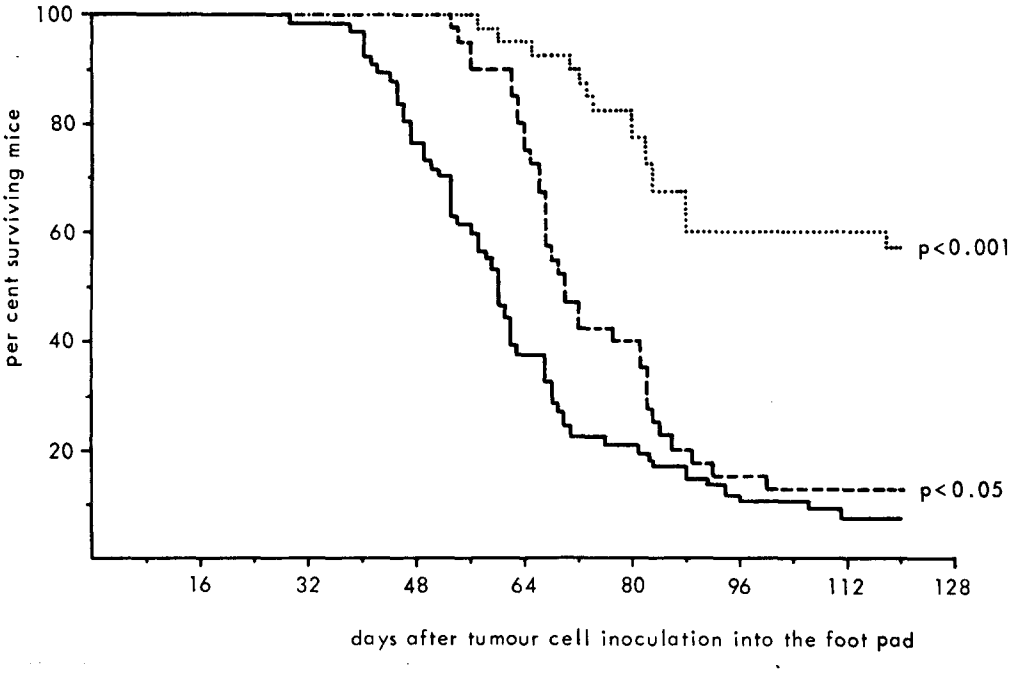

Fig. 7. Survival of mice after treatment with cyclophosphamide on day 1 *or on* days 1–4 *before the surgical removal of a* 2661/*Cy tumour growing in the foot pad. Surgery was performed* 24 days *after s.c. inoculation of tumour cells. Significance in survival was determined in comparison with the control group by log rank test. Control* 5/49 (——); 200 mg/kg *cyclophosphamide* 5/40 (- - -); 4 × 50 mg/kg *cyclophosphamide* 23/40 (. . . . .).

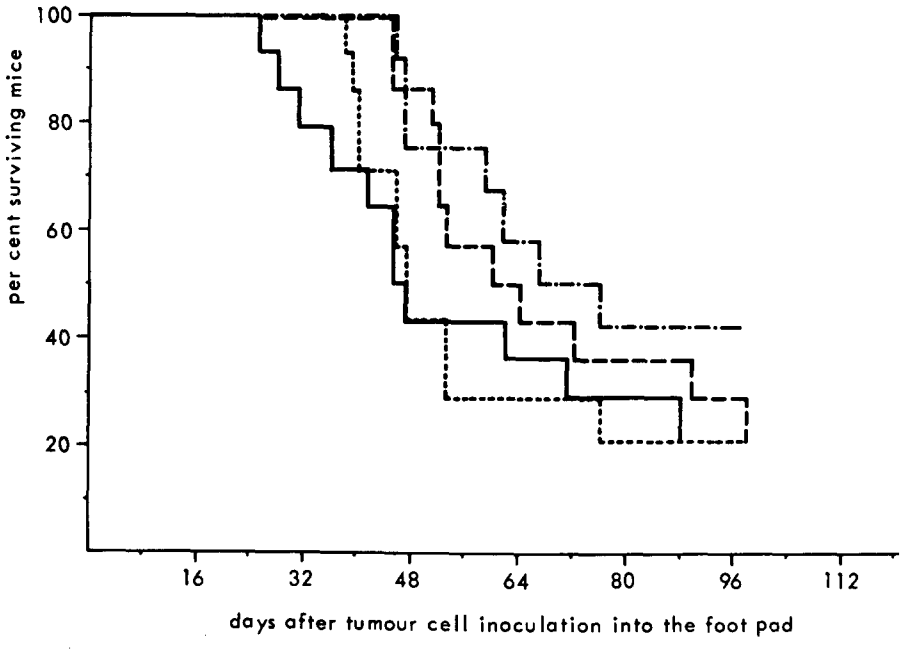

Fig. 8. Effect of various adjuvant chemotherapy schedules, differing in combination of drugs but not in regimen, on survival of mice. The chemotherapy started 3 days *after the surgical removal of a* 2661 *tumour growing in the foot pad. Surgery was performed* 14 days *after s.c. inoculation of tumour cells. The cytostatic drugs used in the combinations were as follows: cyclophosphamide (C); ifosfamide (I); methotrexate (M) and 5-fluorouracil (F). No significant difference in survival was present between the various groups (log rank test). Number of surviving mice: Control* 3/14 (——); *CMF* 5/12 (-.-.-); *IMF* 3/14 (- - -); *MF* 3/13 (. . . . .).

Unfortunately, the effect of treatment with CMF is also not very marked. However, the lack of difference in survival curves seems to indicate that the low efficacy of the adjuvant chemotherapy with CMF is not due to abolishment of the kind of host resistance by cyclophosphamide as seen in the artificial model.

DISCUSSION

The increase in lung nodules in mice pretreated with cyclophosphamide is paralleled by an increased early arrest of radioactively labelled tumour cells in the lung [2, 5]. This finding might suggest that either vascular damage or lung damage will facilitate the early arrest of tumour cells in the lung and result in an increased number of lung nodules. However, the observation that treatment of mice 1–4 days after i.v. inoculation of tumour cells enhances tumour cell survival together with the fact that there is an enhancement of tumour take s.c. [3] or i.m. [6] in pretreated mice argues against this hypothesis.

Another possibility might be that the effect is due to the suppression of the classical immunological resistance (T-cell dependent). However, a number of arguments refutes this hypothesis. The fact that the phenomenon seems not to be T-cell dependent is based on the following results: (a) An increase in the early arrest of cells in the lung within one day after the first contact of the host with the tumour cells [2, 5]; (b) An increase in lung nodules is also present in TIR (thymectomized, irradiated and reconstituted) mice [7]; (c) No influence on lung nodules is seen with antilymphocyte treatment of the host mice, or with attempts at specific immunization with tumour cells [1]; (d) Cyclophosphamide pretreatment does not impair the induction of a specific immune response of a highly immunogenic fibrosarcoma [26]; (e) A similar increase in lung nodules is observed in control mice and mice subjected to whole body irradiation [4, 5, 7]; (f) The increase in lung nodules is extremely high with a non-immunogenic tumour [1–3]; (g) The increase in lung nodules is not influenced by increasing the tumour load or by dose fractionation of cyclophosphamide.

In a recent study [7], however, the suggestion was made that the effect of cyclophosphamide is perhaps partly dependent on non T-lymphocytes. Although we have mentioned that cyclophosphamide apparently decreases host resistance; this host resistance can be due to: (a) A decrease of the non-specific host defense mechanisms which act by active killing of tumour cells; (b) An improvement of the environment in the host for survival of tumour cells which would otherwise had succumbed by a passive cell death [27]. Whatever the mechanism, it has been shown that there are certain similarities between the effect of cyclophosphamide and cortisone acetate. Pretreatment with either agent increased the lung retention of labelled osteosarcoma cells (viable and dead) and of embryonic cells in a similar way [2], (not published for cortisone acetate).

In contrast to this observation, treatment with ifosfamide a cyclophosphamide congener which did not increase the take probability of tumour cells to the same extent as cyclophosphamide, did not affect the lung retention of these cell types. The similarities between the effects of cyclophosphamide and cortisone acetate are of course no proof that both act by a common mechanism. However, the fact that we could at least partly abolish the enhancement effect of posttreatment with cyclophosphamide by pretreatment with cortisone acetate seems to be in favour of this suggestion.

In the studies in which the "survival" of lung colonies was studied in mice pretreated with *C. parvum*, it was observed that cyclophosphamide did not kill the very low number of lung colonies which are present in *C. parvum* pretreated control mice (Fig. 5). Although a slight decrease in the conversion of cyclophosphamide to its active metabolites has been described in *C. parvum* treated mice [28], it seems unlikely that the ineffectiveness of cyclophosphamide is due to total inhibition of its metabolism. Pretreatment with *C. parvum* in control mice increased the host resistance of mice to lung colony formation with a factor of about 1000 (Fig. 5). However, if cyclophosphamide is administered afterwards there seems to be equilibrium between the extent of reversion of this increased resistance and the killing potency of tumour cells at both dose levels. The host resistance after 150 mg/kg cyclophosphamide was similar in control mice and mice pretreated with cortisone acetate or *C. parvum*, if similar cell killing is assumed (Fig. 5). This finding illustrates that the combination of *C. parvum* and cyclophosphamide or cortisone acetate and cyclophosphamide is not always beneficial or negative if you consider the final product, the number of lung colonies. This might be of relevance for the clinical situation in which *C. parvum* or corticosteroids are administered in a number of adjuvant chemotherapy trials.

The results of our studies show that careful evaluation of models permits the recognition of enhanced tumour growth in mice receiving cyclophosphamide. However, it is clear that this enhancement is quantitatively of small importance and a negative effect of adjuvant chemotherapy was not revealed in these studies. Furthermore, there must be marked differences in response in different systems. It has already been pointed out [5] that the lung colony enhancing effect of cyclophosphamide was much more pronounced in our osteosarcoma than in most other systems studied. If this is also true for the effect of cyclophosphamide after tumour cell inoculation, it may explain why this effect was not noted by others who studied the chemotherapy of early lung tumours. Neither Steel and Adams [12] nor Hill and Stanley [22] noted a discrepancy in treatment sensitivity similar to our results with the osteosarcoma and the Lewis lung tumour.

In the spontaneous metastases model with the Lewis lung tumour, it was demonstrated that the growth facilitating effect of cyclophosphamide is also operative if metastases are obtained after spontaneous shedding of tumour cells from a primary tumour. The increase in the number of spontaneous lung metastases was much lower than the increase in lung colonies in artificial lung colony models. However, in the spontaneous model there will be a continuous shedding of tumour cells, and the time at which this process starts, is unknown. Since the enhancement effect of cyclophosphamide is decreasing with intervals of more than 7 days between cyclophosphamide treatment and i.v. cell inoculation [4], the differences in enhancement factors in both models might be due to differences in these intervals. Although pretreatment with cyclophosphamide increased the take probability of i.m. inoculated tumour cells [6], no increase in the local tumour volume was observed in cyclophosphamide pretreated mice. This fact excluded the possibility that differences in primary tumour volume were responsible for the increase in the number of metastases. The metastases facilitating effect was, however, totally abolished if cyclophosphamide is also administered after i.m. tumour cell inoculation. The cytotoxic effects evidently outweighed the metastases facilitating effect.

The studies with the 2661/Cy tumour showed that the suspected insensitivity of this tumour, on the base of growth delay data on flank tumours, did not predict the pronounced effect on the development of metastatic disease. These results seem to indicate that for this tumour either the tumour cells in micrometastases are much more sensitive to cyclophosphamide than in established flank tumours or the actual survival of the cells in the flank tumour is not reflected in the growth delay. In studies with the original "more cyclophosphamide-sensitive" mammary tumour 2661, it was shown that the low efficacy of treatment with CMF adjuvant chemotherapy after surgery of the primary tumour was not due to the metastases facilitating effect of cyclophosphamide.

We can conclude that the metastases facilitating effect of cyclophosphamide could not be demonstrated in our studies if the experiments were designed with a therapeutic set up. In other spontaneous metastases models the only negative effect of cyclophosphamide treatment has been reported in two rat models [29, 30]. Probably, the effect is too small to be identified unless a specific search is made for it. In the clinical application of adjuvant chemotherapy after surgery only one study is described [31] which shows a negative effect of cyclophosphamide if applied after radically operated bronchogenic carcinoma. Nevertheless, it might be useful to analyze this type of resistance in more detail and to search for its mechanism. In addition, it might be opportune to study the mechanism by which adjuvant chemotherapy is of low efficiency in some models.

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