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Adriamycin Effect on the Lewis Lung Carcinoma. Comparison between the Original Line and its Derivative Subline*

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Abstract—*In the present report investigations have been carried out to evaluate the therapeutic response to adriamycin of the Lewis lung carcinoma and of its derivative M1087 subline. The drug was given in a single dose* (14 mg/kg) *at various days post the tumours implantation* (day: 3,5,7,9,12,14,21). *Our findings can be summarized as follows: a different responsiveness to adriamycin was exhibited by the two tumour lines as regards either the primary tumour and the lung metastases. The differences observed can be ascribed, in the former case, to a different growth rate of the cell populations within the tumour. In the latter, many factors can be involved, such as: the different starting-time of metastatic spread in the two lines and the quantitative differences in the total number of lung metastases in the controls.*

INTRODUCTION

MANY authors point out that the existence of tumoural sublines selected within the same tumour represents a useful tool for experimental chemotherapy studies. In fact variants with different potentials for growth rate or metastatic ability should be employed for comparative studies evaluating the response of the different sublines to antineoplastic agents.

Recently, different tumour cell subpopulations derived from single tumour line (BALB/cf C3H mammary adenocarcinoma [1], methylcholanthrene-induced mouse fibrosarcoma (2), UV-2237 fibrosarcoma (3), etc., have been isolated in order to assess a possible different response to antineoplastic drugs.

In our own laboratory extensive research has been carried out to isolate sublines from the Lewis lung carcinoma (3LL). As previously reported [4, 5], we selected for the first time an *in vivo* subline from the 3LL, named M1087. It after transplantation into C57Bl/6 syngeneic mice produced tumours upon i.m. injection of 1×10^3 cells in the 100% of animals; moreover it showed some differences from the parent line, such as the growth rate, the oncogenicity (TD50 value), the starting-time of the metastatic spread.

The purpose of our work was to verify if to the different characteristics, mainly in oncogenicity, observed between original tumour line and M1087 derivative subline would correspond a different sensitivity to a well-known antineoplastic drug, adriamycin (ADM). For this extent we compared the antitumour response of the two lines to a single treatment pursued at various stages of the tumour growth. Results reported below show a different responsiveness to ADM of the two lines of the same tumour, related to the heterogeneity of their malignant characteristics.

MATERIALS AND METHODS

Original 3LL line

Throughout experiments the original line was passaged in 2–3-month-old C57Bl/6 mice; each animal was injected in the leg muscle with 0.1 ml of a suspension containing 2.5×10^5 viable tumour cells. For cell suspension we employed a standard procedure as previously referred [6].

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M1087 subline

This subline was obtained after two *in vitro* selections of cells derived from a biopsy of the spontaneous lung metastases of the original 3LL line. The *in vitro* cell selection was made subculturing single colony showing a high level of *in vitro* plating efficiency (P.E.). The lines obtained with such a process were tested for their ability to give rise to tumours into syngeneic C57Bl/6 mice. After several attempts, *in vitro* established cell lines were obtained which also showed a high oncogenicity. The line C108 [4, 5] which showed the highest P.E. produced the subline M1087. This subline, routinely transplanted i.m., grows in C57Bl/6 mice with macroscopic and histologic characteristics consistent with those reported for the original line. When we performed these experiments the M1087 subline was in the 23rd animal passage. No variation in tumour growth rate and in oncogenicity have been noted up to date (40th animal passage). Tumour cell suspension and transplantation of M1087 subline were performed with the same procedure as for the original line.

TD50 determination

To assess the oncogenicity of both the tumour lines, we determined the TD50 value. Graded inocula of viable tumour cells (without addition of HR cells) were implanted i.m. within a range of concentrations differing by a factor of 2. The data of the limiting dilution assay were analysed 60 days after tumour cells implantation and the TD50 values were calculated by the method of Spearman and Karber [7]. In our experimental conditions, the number of cells which produced 50% takes in recipient mice was 730 (95%c.l. = 949–561) for the original line and 225 (95%c.l. = 330–154) for the M1087 subline.

Drug and treatment

Adriamycin (Adriblastina, Farmitalia, Milan, Italy) was freshly dissolved in saline before i.v. injection. The treatment with a single dose of ADM (14 mg/kg) was carried out on a wide tumour range, from the early stages of the growth (day 3, 5 postimplant) to the latest ones (day 14, 21 postimplant). At least eight mice per group were employed in every experiment (2.5×10^5 viable tumour cells/mouse). The antitumour effectiveness of ADM both on original line and on the M1087 subline was estimated by the following, usual parameters: (i) tumour growth inhibition (T/C), (ii) tumour growth delay (T–C, days) to a predetermined size according to Lloyd [8] (see Table 1), (iii) metastasis reduction (T/C) by counting the lung nodules fixed in a Bouin's solution with the aid of a dissecting microscope, (iv) percent increase in host life span (%ILS).

Surgery

Different days after both tumour lines implantation (day 1, 4, 5, 7, 8, 10) a surgical amputation of the tumour-bearing leg was performed with the same procedure as previously described [6].

Statistical analysis

Statistical significance of the differences between values of controls and treated groups was assessed by Student's *t*-test.

RESULTS

In Fig. 1. it is possible to examine the effect of treatment on the growth rate both of original line and of M1087 subline of 3LL tumour, compared with their respective controls. As can be seen, the patterns of the tumour growth after ADM reveal a different sensitivity between the two lines. In particular, the M1087 subline exhibits a greater antitumoural response over the original line, more evident when the treatment is pursued on late stages of the tumour growth. In fact, as reported in Table 1, when ADM is administered on day 9, 12 or 14 after tumour implantation, the values of the tumour growth inhibition for the M1087 subline are significantly lower than that for the original line (T/C: 0.43–0.45–0.5 vs 0.71–0.61–0.65, respectively).

More evident quantitative variations were observed between the two lines for the growth delay parameter. In particular the M1087 subline treated on late stages of the tumour growth shows a T–C value about 2.5/3 times higher than that of the original line.

Also the %ILS of treated mice vs controls shows a different trend in the two tumour lines (see Table 1), even if the values are not significantly high, due to the well known weak effect of ADM on i.m. implanted 3LL [9].

As mentioned above, some differences are exhibited by both the tumour lines in their spontaneous metastatic ability. In fact, data obtained from previous research in our labo-

Table 1. *Effect of adriamycin on original Lewis lung carcinoma and M*1087 *derivative subline*

Experimental group	Day of treatment	Effects of primary tumor: Average t.w. (g ± S.E.) original	Average t.w. (g ± S.E.) M1087	T/C original	T/C M1087	Growth delay‡ (days) original	Growth delay‡ (days) M1087	Effect on metastases: T/C§ original	T/C§ M1087	%ILS‖ original	%ILS‖ M1087
Control		5.00 ± 0.25	7.01 ± 0.68								
ADM, 14 mg/kg	3	2.87 ± 0.31*	3.05 ± 0.35*	0.57	0.43	6.5	6	0.85	0.48	44	30
	5	3.52 ± 0.68*	n.e.	0.70	n.e.	2.5	n.e.	0.54	n.e.	3.1	n.e.
	7	3.16 ± 0.22*	5.17 ± 0.26†	0.63	0.74	2.5	2	0.50	0.61	14.1	16.4
	9	3.58 ± 0.17*	3.05 ± 0.18*	0.71	0.43	2.5	6	0.53	0.54	1.0	12
	12	3.04 ± 0.04*	3.13 ± 0.13*	0.61	0.45	1.5	4.5	0.08	0.57	−3	11
	14	3.25 ± 0.27*	3.49 ± 0.47*	0.65	0.49	1.5	5	0.14	0.28	−0.4	25
	21	n.e.	6.18 ± 0.62	n.e.	0.88	n.e.	3	n.e.	0.46	n.e.	20

*$P < 0.01$; †$P < 0.05$ vs respective controls (Student's *t-test*).

‡In both lines, for the tumours in early (d. 3, 5), in first palpation (d. 7) and in advanced stage (d. 9, 12, 14), the time to reach 500 mg, 1000 mg, 2500 mg respectively, was taken.

§The average number of lung nodules in the Control group was 17 ± 3.9 (original line) and 48.5 ± 4.3 (M1087 subline).

‖The MST of the Controls was 22 ± 1.7 days (original line) and 25.2 ± 2.1 days (M1087 subline).

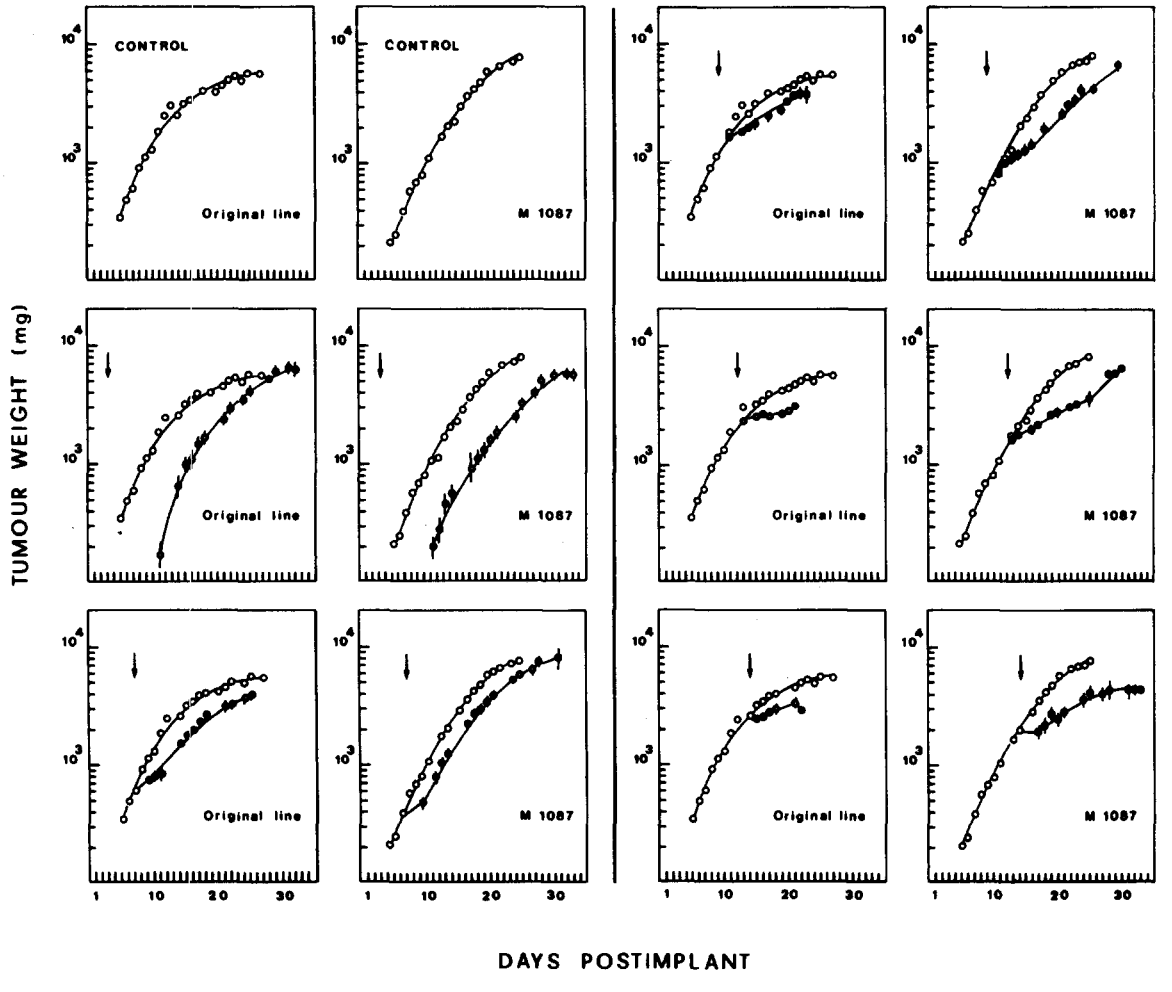

Fig. 1. *Effect of a single dose of ADM* (14 *mg/kg*) *on the original* 3*LL and its M*1087 *derivative subline. Each point represents the mean value of eight animals* (○ *Controls,* ○ *ADM treated); when not indicated, the S.E. squares are enclosed inside the symbols. The arrows indicate the day of treatment.*

ratory showed a clear quantitative difference in the production of spontaneous metastases between the two lines (unpublished data). Besides, the surgical removal of the primary tumour at various stages of the growth, was able to produce a higher percentage of long-term survivors in original 3LL-bearing mice. The results of these experiments reported in Fig. 2, could demonstrate a different starting-time of the metastatic cells spreading from the original or from the derivative M1087 line.

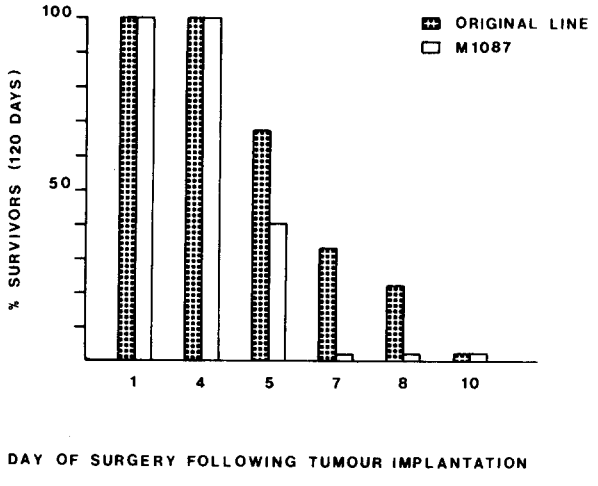

Fig. 2. Comparison of survivors after the surgical amputation of the primary tumours, performed on day 1,4,5,7,8 *or* 10 *postimplant. At least six animals per group were employed.*

Therefore, we carried out further experiments to assess if the difference in sensitivity to ADM observed in the primary tumour of both the lines, would maintain also in the secondary tumours. Results reported in Table 1 show a different metastasis reduction between the two lines after ADM treatment. In fact, the metastases from the original 3LL exhibit a sensitivity to the drug progressively greater (T/C day 3: 0.85 T/C day 14: 0.14) whereas the metastases from the M1087 subline display a constant response in all the stages of the treatment.

DISCUSSION

The aim of this study was to see whether two cell lines derived from the same tumour (3LL) and differing in some malignant characteristics, would show a differential sensitivity to the treatment with ADM. It emerges from the present work that this is the case.

As previously pointed out, by examining the response to ADM of the primary tumours it is possible to see that the drug effect is quite similar in the earliest stages of treatment for both the lines, whose sensitivity is significantly high. Conversely, in the latest stages of treatment the drug effect on the original line markedly diminishes, whereas in the M1087 subline it elicits a constant, satisfactory level. This is particularly evident for the 'growth delay' parameter. We believe that the different response of the primary tumours of the two lines to ADM could be primarily ascribed to their different growth rate. In fact, the M1087 subline growth curve exhibits a more exponential trend in comparison to that of the parent line, which is evident until the latest stages of the growth (see Controls of Fig. 1). Therefore our findings indicate that the better activity of ADM on the M1087 derivative subline could be related to the different proliferative state rather than to a different, intrinsic sensitivity of the two lines. If this were the case, the difference in responsiveness between the two tumours should keep constant during all the stages of the treatment.

As regards the therapeutic effect of ADM on metastases, the original line elicits a response whose level progressively increases from the earliest stages to the latest ones. In particular, markedly high values are scored when the treatment is delayed to 12th or 14th day postimplant, in good agreement with the data reported in literature [10, 11]. Conversely, the response shown by the metastases of M1087 subline keeps constant during all the stages of the treatment. We believe that many factors could be involved to explain these results, among those; the delayed spread of metastatic cells from the original line and the lesser total number of metastases produced by the parent line (17 ± 3.9 orig. l. vs 48.5 ± 4.3 M1087 l., 21 days after tumour implantation). The analysis of our findings indicating a different effect of ADM on lung nodules of the two lines is under way. It is mainly based on two hypotheses: (1) the presence of an intrinsic cell resistance (or sensitivity) in the two lines of the same tumour; (2) a different ADM pharmacodynamics between the two tumour subpopulations. We believe that these preliminary data are interesting since they confirm the usefulness of the employment of sublines isolated from the same parent tumour, in cancer chemotherapy studies.

Because of the great interest of this kind of

experimental research we are extending our studies on further derivative sublines from the original 3LL, in order to elucidate the relation between pharmacodynamics, growth rate of tumours and the response to chemotherapeutic agents.

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