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METHODS OF IMMUNOGENICITY TESTING OF SOLID TUMOURS IN MICE: A COMPARISON OF THE RESULTS

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THIS report is concerned with the influence of the host (C57BL/Ka) on tumour take (Lewis lung carcinoma (LL)) after various immunization procedures. Two types of experiments were performed: Lung colony assays and endpoint dilution studies. Immunization was carried out with either irradiation killed tumour cells or with a growing tumour mass. The latter method of immunization may result in immunity induced by viable tumour cells which can be investigated after excision of the tumour and is therefore the most relevant experimental model for chemo-immunological adjuvant therapy.

Results for mice immunized with heavily irradiated LL cells and challenged either i.v. with viable LL cells or s.c. with serial dilutions of LL cell suspensions are presented in Table I. The number of lung colonies in immunized mice is smaller than in control mice, indicating host resistance to tumour take. A discrepancy can be demonstrated between the two methods of investigation: the lung colony assay is much more sensitive than the classical endpoint dilution assay.

TABLE I.—*The effect of immunization with heavily irradiated cells on the number of lung colonies and number of cells needed for 50% Lewis lung tumour take*

Procedure	Endpoint dilution assay TD_{50}	Lung colony assay Colonies per mouse
Non-immunized	$5{\cdot}5 \cdot 10^4$	115 (79–200)
Immunized	$8 \cdot 10^4$	11 (1–24)

Mice were immunized by s.c. injection of 10^6 irradiation-killed LL tumour cells on Days 1 and 15. For the endpoint dilution assay, non-immunized and immunized groups of 4 mice were s.c. challenged at Day 29 at 4 different sites. Tumour take was recorded up to 60 days after challenge. For the lung colony assay, non-immunized ($n=11$) and immunized ($n=8$) animals were challenged with 10^6 LL cells. The animals were sacrificed 2 weeks later and the number of lung colonies counted.

Table II shows the influence of postoperative immunity on the number of colonies and on the number of LL cells needed for 50% tumour take: although the number of lung colonies in control animals differs from one experiment to another, the same pattern as in Table I is observed. In contrast to immunization with irradiation killed LL cells (Table I), this method of immunization increases the TD_{50} significantly.

In conclusion, the LL carcinoma inoculated into C57BL/Ka mice was proved to be immunogenic. An opposite conclusion might have been reached if relatively insensitive methods of investigation had been used, *e.g.* TD_{50} measurements after injections of heavily irradiated cells.

TABLE II.—*The effect of immunization induced by a growing tumour on the number of colonies and number of cells needed for 50% Lewis lung tumour take*

Procedure	Endpoint dilution assay TD_{50}	Lung colony assay Colonies per mouse
Non-immunized	$4 \cdot 10^4$	33 (9–36)
Immunized	$3 \cdot 10^5$	4 (9–0)

Mice were inoculated s.c. or not with LL tumour cells. The tumour was excised from Nembutal anaesthetized animals on Day 10. Control mice were sham-operated. For the endpoint dilution study, non-immunized and immunized groups of 4 mice were challenged s.c. at day 12 at three different sites. Tumour take was recorded up to 90 days after challenge. For the lung colony assays, non-immunized ($n=10$) and immunized ($n=10$) animals were challenged with 10^6 LL cells. Lung fixation was performed 15 days after i.v. challenge. Historical control experiments did not show a significant number of spontaneous lung metastases or local tumour recurrences.