

# Radiocurability of a transplantable murine sarcoma as influenced by immune competence of the host and adjuvant active specific immunotherapy

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The immunization induced in CBA mice by allogenic transplantation of sarcoma J was evaluated through the rejection rate of a subsequent isologous tumour graft. Female animals appear to be significantly much more competent at various stages of sarcoma growth as after their definite cure by radiation or surgery. A difference of response according to the sex is also present with regard to the auto-immune reaction evoked by tumour irradiation. A marked discrepancy in radiotherapy efficiency is linked to the disparity of immune behaviour, emphasizing the fact that an immunologically competent host constitutes an important factor of tumour radiocurability. In male recipients, an adjuvant active specific immunotherapy potentiates tumour response to a single dose of radiations. On the contrary, the adjunct treatment may display a detrimental effect in animals which already possess a high degree of anti-tumour immunization.

The immunological reactivity of the host seems to influence tumour radiocurability, at least to some extent. The response to a single dose of radiations depends on the status of immunosuppression or immunostimulation induced in the animal bearing a tumour. Indeed, a preliminary whole-body irradiation or the administration of corticoids reduce the efficiency of the radiotherapy [3, 13, 15]. On the contrary, an active specific immunotherapy is likely to display an adjuvant effect [2, 4, 8].

Our experimental model consists of a transplantable murine sarcoma which proved to evoke quite a different immunological response according to the sex of the recipient. We assessed whether tumour radiocurability is influenced by the sex-linked disparity of immune behaviour as also by an active specific immunotherapy.

## Materials and methods

### 1. Tumour

Sarcoma J has spontaneously originated in a C<sub>57</sub> Black mouse [1]. By subcutaneous transplantations at regular intervals, we maintain a tumour strain in allogenic situation in CBA mice. The following

experiments took place between the 10<sup>th</sup> and 90<sup>th</sup> transplantation.

### 2. Animals

We used CBA mice from our own breeding the racial purity of which is checked regularly by skin crossgrafting. The mean age of the experiment animals is about 2 months. They are fed with standard diet pellets and tap water ad libitum.

### 3. Tumour graft

A mouse bearing a tumour strain is killed by cervical dislocation. Tumour pieces of 2 mm size are aseptically prepared. By means of a trocar they are inoculated intradermally in the medio-dorsal region of the animals.

### 4. Measure of tumour growth

Every two days the greater (a) and the smaller (b) diameters of the tumour are measured with a  $\frac{1}{10}$  caliper.  $\sqrt{ab}$  means the diameter of the circle whose surface equals to the section through the plan of tumour growth. For each animal the successive values of  $\sqrt{ab}$  are plotted against the time from tumour inoculation. These values are treated on a computer program according to 3 different growth patterns (linear progression, exponential and Gompertz functions).

### 5. Tumour radiotherapy

A single dose irradiation by Cobalt gamma rays takes place on day 8<sup>th</sup> of tumour growth. A special

	male mice	female mice
mean growth coefficient	0.9 ± 0.22	0.86 ± 0.16
mean survival time (days)	24 ± 5.4	24 ± 6.7

Table 1. Parameters of tumour growth. Mean angular coefficient of tumour growth and animals mean survival time (lots of 50 mice were used for each sex).

immobilization device facilitates the exposure of the target volume without anesthetizing the animal. The determination and expression of the tumour-dose have been previously described [4].

### 6. Surgical treatment

A lot of animals were cured by ligation of the tumour pedicle 8 days after sarcoma inoculation. Surgical excision of the tumour within the same interval was made in another lot.

### 7. Evaluation of anti-tumour immunity

Mice bearing an actively growing dorsal tumour and mice cured of a primary sarcoma receive a tumour inoculation on their ventral side. This challenge graft contains about 10<sup>6</sup> tumour cells; it is periodically checked, and rejections or takes are tabulated.

### 8. Adjuvant active specific immunotherapy

Isologous tumour pieces are aseptically removed from a tumour strain grafted mouse and placed in a sterile saline physiologic solution kept at room temperature. These fragments are irradiated to complete cellular inactivation by a 10.000 rd dose and implanted intradermally in the ventral region of sarcoma bearing mice. The adjunct treatment takes place 4 hours before primary tumour radiotherapy.

## Results

### 1. Growth of sarcoma J in CBA mouse

Under strict aseptic conditions the percentage of graft takes amounts to 100. Tumour development is purely local without deep infiltra-

tion or visible metastases; ulceration is unfrquent. The tumour is palpable on day 4<sup>th</sup> after inoculation; it becomes measurable from day 6<sup>th</sup>.

The growth of sarcoma J in CBA mouse occurs following a linear progression up to the death of the animal. There is no temporary variation or terminal slackening off, and spontaneous regression is not observed.

The mean growth coefficient calculated from individual angular coefficients is 0.9 for male mice and 0.86 for females. The median survival time is 24 days in both sexes (Table 1).

### 2. Immunization of the host during tumour growth

Eight days later than the primary graft 40% of male mice and 80% of female mice reject a second isologous tumour graft. In the case when the challenge graft is made on day 15<sup>th</sup> of the sarcoma growth, rejection rates fall respectively to 26% and 50% (Table 2).

### 3. Auto-immune reaction induced by tumour irradiation

The rejection rate of challenge-graft rises up to 65% in male mice and to 89% in females eight days after having delivered 4.000 rd to the primary tumour. In controls bearing unirradiated tumour, rejection rates are respectively 26 and 50 per cent (Table 3).

days after primary graft	rejection rate of challenge graft	
	male mice	female mice
8	40% (21/52)	80% (44/54)
15	26% (18/68)	50% (30/60)

Table 2. Immunization of the host during tumour growth. Rejection rate of a second isologous tumour grafted eight and fifteen days later than primary sarcoma inoculation (results significantly different: P < 0,01).

	rejection rate of challenge graft	
	male mice	female mice
day(8) after tumour irradiation	65% (31/48)	80% (47/53)
controls with unirradiated tumour	26% (18/68)	50% (30/60)

Table 3. Auto-immune reaction induced by tumour irradiation. Rejection rate of a second isologous tumour grafted eight days after primary sarcoma irradiation (4.000rd) (results significantly different:  $P < 0,01$ ).

#### 4. Immunity of cured animals

Two months after curative treatment (ligation, excision or irradiation) female mice offer a maximum rejection rate of challenge-graft. In males, surgery or low-dosage radiotherapy (4.000 rd) immunize only in the proportion of one to three. The percentage of resistant mice is twice as high when 10.000 rd are delivered to the tumour (Table 4).

#### 5. Tumour radiocurability

Several opportunities occur according to the radiation dosage:

- a) tumour growth stops for 3–4 days, afterwards it resumes following an angular coefficient next to the previous one and the animal gets death from its tumour.
- b) after a stabilization period, the tumour regularly diminishes and disappears. A local recurrence nevertheless occurs within 4 weeks after the irradiation.
- c) the local control lasts 2 months after irradiation: the animal is, for good, free of recurrence and may be considered as cured.

Table 5 shows the degree of sarcoma J radiocurability according to the host's sex and radiation dosage. Lots of about fifty animals were used for each dose. Fifty per cent of

female mice are cured by a tumour dose of 2.000 rd, whereas an equal dose only provides a negligible recovery rate in males.

#### 6. Effect of adjuvant active specific immunotherapy on tumour radiocurability

Irradiated tumour isografts greatly potentiate the response of sarcoma J to a single dose of radiations when the host is a male mouse. On the contrary the immunotherapy proves to be of no value with females and besides, to some extent, it appears to be unfavourable (Table 6).

#### Discussion

Owing to the possession of neo-antigens, a primary tumour can be compared to a homograft in its host [9]. An allogenic tumour transplantation system is, therefore, suitable as an experimental model of host-tumour relationships [11]. Nevertheless one should keep in mind that, in this case, a double antigenic determinant operates. The immunological response to tumour specific transplantation antigens adds its effects to the homograft reaction against tissue histocompatibility antigens.

curative treatment	rejection rate of challenge graft	
	male mice	female mice
ligation	20% (5/25)	85% (22/26)
excision	33% (10/30)	88% (27/31)
RxTh (4.000 rd)	33% (9/27)	100% (30/30)
RxTh (10.500 rd)	60% (21/35)	100% (36/36)

Table 4. Immunity of cured animals. Rejection rate of a second isologous grafted two months after curative treatment of the primary sarcoma (results significantly different  $P < 0,001$ ).

tumour dose (Rad)	recovery rate	
	male mice	female mice
600	0% (0/45)	2% (1/48) ( $\alpha$ )
1.300	2% (1/46)	42% (22/52)
2.000	2% (1/46)	49% (23/47)
4.000	20% (10/49)	67% (36/54)
8.000	81% (39/48) ( $\alpha$ )	87% (39/45) (a)
10.500	83% (39/46)	98% (44/45)

Table 5. Sarcoma J radiocurability. Influence of graded single doses on recovery rate (irradiation on day 8th of tumour growth,  $\gamma$  rays of cobalt 60) [results significantly different  $P < 0,001$  unless marked( $\alpha$ )].

In our own experimental system host's sex influences immunization in a clear cut way. At various stages of sarcoma J growth, immune reactivity of female mice appears to be significantly higher than that of males. Tumour growth pattern, however, looks fairly independent of sex and so is not necessarily a good correlate of immunological response [5]. The auto-immune reaction evoked by tumour irradiation which was already point-

ed out in a previous publication [4] is present in both sexes, but female mice again achieve a better status of immunization. In animals cured of a primary tumour the specific resistance towards a subsequent sarcomatous graft is also more important in females.

Several reports confer a better immune competence to female animals [6, 7]. More peculiarly as far as sarcoma J is concerned, Betz [1], in isogenic system, noticed twice as much spontaneous regressions in females as in males; castration and oestradiol administration to the latter increased the regression rate.

A better phagocytic activity displayed by macrophages might be the reason for this difference, oestrogens being known as potent stimulants of the reticulo-endothelial system [12, 14].

Our work emphasizes the fact that an immunologically competent host eventually constitutes an important factor of tumour radiocurability. A marked discrepancy in radiotherapy efficiency is indeed linked to the disparity of immune behaviour. The difference in response is mainly noticeable as far as low doses of radiations are concerned. In this range, tumour eradication results from several interacting factors: direct effect on neoplastic cells, conjunctivo-vascular modifications of tumour bed and influence of immu-

tumour dose (Rad)	recovery rate			
	male mice		female mice	
	RxTh	RxTh+AASI	RxTh	RxTh+AASI
600	0% (0/45)	0% (0/46)	2% (1/48)	2% (1/43)
1.300	2% (1/46) ( $\alpha$ )	14% (6/42) ( $\alpha$ )	42% (22/52)	39% (18/46)
4.000	20% (10/49) ( $\beta$ )	40% (21/53) ( $\beta$ )	67% (36/54) ( $\gamma$ )	50% (25/50) ( $\gamma$ )

Table 6. Effect of adjuvant active specific immunotherapy (AASI) on tumour radiocurability. Influence of irradiated tumour isografts adjunct on the response of Sarcoma J to a single dose of radiation [differences in results statistically significant: ( $\alpha$ ) =  $P < 0,001$ ; ( $\beta$ ) =  $P < 0,03$ ; ( $\gamma$ ) =  $P < 0,08$ ].

nological defences. As the dose is increasing, the lethal direct action becomes preponderant on other mechanisms of cellular inactivation: the part of the immunization level diminishes then proportionally.

A few observations may be found in agreement with the results reported here. Using a C<sub>3</sub>H mouse mammary carcinoma, Cohen [2, 3] pointed out that immunosuppression (whole-body irradiation or cortisone administration) rose the tumour control dose in 50 per cent of the animals (TCD<sub>50</sub>) from 5.660 to 7.500 rd. Experiments of van den Brenk [15] demonstrated that the TCD<sub>50</sub> of Ehrlich carcinoma was 3.5 times higher in mice totally irradiated with 250 rd than in controls. Powers [13] determined the variations of TCD<sub>50</sub> for Gardner lymphosarcoma transplanted in its strain of origin. It equals 1.500 rd in normal hosts, 3.200 rd in immunosuppressed animals and falls to 400 rd in mice cured of a previous isologous tumour.

On the ground of experimental data collected in mice with LSA lymphoma, Maruyama [10] inferred a mathematical model of radiological inactivation where intrinsic response of cells to radiation plays a part as well as immune competence of the host.

An active specific immunotherapy is likely to influence tumour response to a single dose of radiations. Cohen [2] mentions the "radiosensibilization" obtained towards a C<sub>3</sub>H mouse mammary carcinoma by implantation of the host with radiation attenuated tumour tissue. Under these conditions 4.200 rd led to

a cure in 50 per cent of mice as opposed to 0.1% in controls.

Haddow and Alexander [8] greatly improved the effect of 2.000 rd on a benzyrene induced fibrosarcoma by inoculation of rats with irradiated tumour autografts prior to radiotherapy.

In our experimental system immunotherapy proves to be beneficial or unfavourable according to the sex of the host. Male mice which naturally are weakly immunized take advantage of an extra-supply of antigenic material and mount a response the effects of which potentiate the irradiation. It is worthwhile mentioning that a threshold exists: too low a dose of radiation which by itself does not lead to tumour eradication cannot be boosted by immunotherapy. On the contrary, the adjunct treatment is of no value in females which already possess a high degree of immunization. The results we report here are even compatible with the concept that, in this case, a reverse action may occur, possibly through an enhancement phenomenon.

Until this effect has not been accurately delineated it seems idle to speculate on the exact mechanism. Results of this kind should, however, focus the attention on risks that might bear an active specific immunotherapy when administered without taking into account the immune status of the host.

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#### **Strahlenheilbarkeit eines übertragbaren Murinsarkoms, das durch die Immunitätsfähigkeit des Empfängers und die aktive, spezifische und adjuvante Immunotherapie beeinflusst ist**

Die in CBA-Mäuse induzierte Immunisierung bei allogener Transplantation des J-Sarkoms wurde durch die Ausstoßrate einer späteren isologen Tumor-Übertragung untersucht. Weibliche Tiere scheinen empfindlicher zu reagieren in den verschiedenen Stadien des Sarkom-Wachstums als nach ihrer durch Bestrahlung oder Operation bewirkten Heilung. Ein Unterschied in der Reaktion gemäß dem Geschlecht besteht auch in bezug auf die Autoimmunitätsreaktion, die durch die Bestrahlung des Tumors hervorgerufen wurde. Eine deutliche Diskrepanz in der Wir-

kung der Strahlentherapie ist mit der unterschiedlichen Immunreaktion verbunden und betont die Tatsache, daß ein immunologisch fähiger Empfänger einen wichtigen Faktor für die Tumor-Strahlenheilbarkeit in sich enthält. Bei den männlichen Empfängern beeinflusst eine aktive, spezifische und adjuvante Immunotherapie die Antwort des Tumors auf eine einfache Strahlendosis. Vielmehr kann die zusätzliche Behandlung eine schädliche Wirkung auf die Tiere hervorrufen, die schon eine hohe Tumor-Immunsierungsrate besitzen.

**Influence de la compétence immunitaire de l'hôte et d'une immunothérapie active spécifique adjuvante sur le degré de radiocurabilité d'un sarcome murin transplantable**

L'immunisation induite chez la souris CBA lors de la transplantation allogénique du sarcome J a été testée par le taux de rejet d'une greffe tumorale isologue ultérieure. Les animaux femelles apparaissent significativement beaucoup plus compétents aux différents stades de la croissance du sarcome ainsi qu'après leur guérison par irradiation ou chirurgie. Une différence de réponse liée au sexe existe aussi en ce qui concerne la réaction auto-immune provoquée par l'irradiation de la tumeur. Une variation marquée d'efficacité de la radiothérapie est liée à la disparité de comportement immunitaire et souligne le fait qu'un hôte immunologiquement compétent constitue un facteur important de la radiocurabilité d'une tumeur. Chez les animaux de sexe masculin, une immunothérapie active spécifique adjuvante potentialise la réponse tumorale à une dose unique de radiations. Au contraire, ce traitement complémentaire peut avoir un effet néfaste chez les animaux qui possèdent par avance un haut degré d'immunisation anti-tumorale.

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