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Resistance to Growth of 3-Methylcholanthrene(3-MC)-Induced Primary Tumours in BALB/c Mice following Pregnancy or Immunization Against Foetal Tissues

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Abstract—*The induction and subsequent growth of 3-MC-induced primary tumours was examined in syngeneic BALB/c mice which had given birth to a single litter of young and in other mice pretreated with* 13–14 day *gestation foetal tissues. Both groups showed no significant difference from age matched virgin controls in the frequency of the appearance of palpable tumour nodules following subcutaneous (s.c.) injection of* 66 μg *of 3-MC dissolved in olive oil. The highest incidence of tumours in all groups occurred at about* 16–18 weeks *following the carcinogen treatment. After the appearance of palpable tumours there was a significant difference in the rate of regression of the primiparous tumours compared to the control group tumours. Only* 4% (1/24) *of the control group tumours regressed after appearing in contrast to* 33% (9/27) *primiparous group tumours and* 15% (4/26) *foetal-tissue-treated group tumours. The possibility is considered that pre-existing sensitivity to oncofoetal antigens assisted the tumour-specific immune response in the rejection of nascent tumours.*

INTRODUCTION

CERTAIN FOETAL antigens reappearing in tumours can stimulate syngeneic lymphocytes and transform them into cytotoxic cells capable of killing normal foetal cells as well as tumour cells expressing such antigens. This has been shown in a number of laboratories by *in vitro* techniques [1–4]. There is also increasing evidence that under certain conditions foetal antigen-sensitive animals develop the ability to inhibit, to a limited extent, tumours of syngeneic origin following tumour challenge or following cell transfers [5–7]. Furthermore, it has been reported that parity in rats [8] or pre-immunization using foetal tissues in mice [9, 10] leads to a degree of protection against the development of chemically-induced primary tumours.

In a previous report [7] evidence was presented that BALB/c mice which were sensitized against foetal antigens during pregnancy developed T cells that were weakly but significantly inhibitory to syngeneic MC-induced tumour cells *in vivo*. This communication presents observations on the rejection—possibly immunologically mediated—of 3-MC-induced primary tumours by BALB/c mice following pregnancy or following active immunization with foetal tissues.

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MATERIALS AND METHODS

These have been reported previously [7] and only departures from these procedures will be detailed.

Tumour induction

Tumours were induced by s.c. injection of 3-MC dissolved in olive oil. A solution containing 66 μg of 3-MC in 0.05 ml was carefully injected s.c. into the shaved flank using a 1.0 ml tuberculin syringe and 24 g needle.

Tumour detection

The flank into which 3-MC was injected was kept shaved so that appearance of

any tumours could be readily detected. Palpation at the site of 3-MC injection was regularly carried out starting 10 weeks following the carcinogen treatment. Clearly palpable nodules were recorded as positive for tumours. Such nodules always arose as small visible lumps and invariably only one such nodule per mouse was detected. Mice which had their tumours regressed and others which developed no detectable tumours were kept under observation for a total period of over 45 weeks.

Pre-treatment of experimental mice

Female BALB/c mice 3–3½ months of age were mated with isogeneic males. Following a single pregnancy those giving birth to three or more young were selected and 30 of the animals that had given birth to a litter within 3 weeks of each other were treated with 3-MC, 14 days following the latest litter birth (group 1).

Thirty virgin mice of similar age were "immunized" as follows, on four successive occasions, prior to 3-MC treatment (group II). On two occasions 13–14 day gestation embryos were trypsinized and cells cultured overnight. The cells were harvested by re-trypsinizing for 15 min, washed twice in Eagles Minimum Essential Medium (MEM) and treated for 20 min with 0.025% glutaraldehyde. The cells were washed twice in MEM prior to injection s.c. into mice. Each received approximately 3×10^7 and 2×10^7 cells, respectively, on the two occasions. On two further occasions the mice were injected i.p. with thoroughly homogenized 13–14 day gestation embryos, each receiving approximately half an embryo on each occasion.

Thirty normal-age-matched virgin mice set apart at the beginning of the experiment were used as controls (group III). All three groups of mice were treated with 3-MC 14–35 days following litter birth in the first group, or 20 days following the immunization schedule in the second group.

Statistical evaluation of data

The significance of the differences between groups with respect to proportions developing palpable tumours or regressing developed tumours was assessed by Fisher's Exact Test for 2×2 tables. The levels of significance are shown in Table 1 and in the text where appropriate.

RESULTS

All three groups were observed for their frequencies of appearance of palpable tumours and for further development or suppression of the tumours during a total period of over 45 weeks from the time of 3-MC treatment. Table 1 shows that in all three groups development of palpable tumours steadily increased during the 16th to 18th weeks by which time maximal proportions of mice developed tumours in all three groups. Thus 27/30 primiparous animals (group I) and 26/30 "immunized" animals (group II) had palpable tumours after 16 weeks while 24/29 controls (group III) developed tumours after 18 weeks. One of the latter group died after 16 weeks without signs of tumour and was excluded from the experiment. There was no significant difference either in the rate of appearance of the tumours or in the maximal proportions developing tumours between groups. Observation over a total period of 45 weeks showed no further development of tumours in the remaining mice. In group III mice the proportion developing tumours remained the same until the 30th week but by the 35th week one of the tumours regressed leaving 23 with tumours. In contrast 9/27 tumours in the primiparous group and 6/26 tumours in the actively immunized group regressed within the following 7–10 weeks. However, two of the regressed tumours in the latter group either resumed growth or new tumours arose in these two tumour regressed mice and the proportion freed of tumours after 35 weeks was 4/26. In the primiparous animals the 9 tumours that regressed remained impalpable during the following 19 weeks of observation.

The difference between controls (group III) and primiparous (group I) animals in respect of the proportions which regressed tumours was significant at all intervals after the 23rd week and this significance reached the 1% level at 26 weeks ($P=0.0031$) and at 30 weeks ($P=0.0031$). Towards the end of the period of observation, significance fell to $P=0.019$ due to the regression of a single tumour in the controls. Between the "immunized" (group II) and the control group (group III) a significant difference ($P=0.028$) was detected only at 23 weeks. In all three groups the tumours that did not regress enlarged during the period of observation. It was assumed that 3, 4 and 5 mice, respectively, of the primiparous, "immunized" and control groups that did not develop palpable tumours during the

Table 1. *Resistance to the growth of* 3-*methylcholanthrene*(3-*MC*)-*induced primary tumours in female BALB/c mice given birth to a single litter or immunized to foetal tissues*

Group	Treatment	Proportion of mice in group with tumours or tumours regressed	Weeks post s.c. injection of 3-MC									
			10	13	16	18	21	23	26	30	35	45
I	Given birth to a single	With tumours	12/30*	12/30	27/30	24/30	22/30	20/30	18/30	18/30	18/30	18/30
	litter of 3 or more young	Tumours regressed	—	—	—	3/27†	5/27	7/27	9/27	9/27	9/27	9/27
II	Immunized against 13–14 day	With tumours	9/30	9/30	26/30	23/30	22/30	20/30	21/30	21/30	22/30	22/30
	gestation foetal tissues × 4	Tumours regressed	—	—	—	3/26	4/26	‡6/26	5/26	5/26	4/26	4/26
III	Normal virgin mice	With tumours	16/30	16/30	23/30§	24/29	24/29	24/29	24/29	24/29	23/29	23/29
		Tumours regressed	—	—	—	—	0/24	0/24	0/24	0/24	1/24	1/24

*No. of mice with palable tumours/total No. of mice in group.
†No. of mice with regressed tumours/total that developed tumours.
‡Two of the 6 tumours that regressed resumed growth later.
§One mouse died without detectable tumour.
A significant difference existed between proportions regressing tumours as follows: Primiparous vs controls at :23 weeks $P=0.015$; 26 weeks $P=0.0031$; 30 weeks $P=0.0031$; 35 weeks $P=0.019$. Immunized vs controls at: 23 weeks $P=0.028$.

period of observation were unlikely to have developed and rejected clinically undetectable tumours, but this possibility cannot be totally excluded.

DISCUSSION

These observations indicate that experience of pregnancy in BALB/c mice confers on them a degree of protection against subsequent outgrowth of nascent tumours. Evidence for a similar effect following active immunization using 13–14 day gestation foetal tissues, according to the schedule utilized here, is less apparent. However, recent observations by other investigators [9–12] suggest that inappropriate presentation of antigen may account for these results. The interesting finding from the present investigation, however, is the observation that primary tumours which had become palpable could be regressed and were possibly totally rejected in a significant proportion of the primiparous (group I) mice as compared to age-matched virgin controls.

Moon [8] reported that a single pregnancy in rats prior to feeding them with 7,12-dimethylbenz(a)anthracene resulted in a significant decrease in the incidence, as well as an increase in the time of appearance of the first palpable tumours. Parmiani and Lembo [13] had shown similarly that multiparous C3H/f mice developed tumours less frequently and after a longer latent period following implantation of 3-MC pellets than did age-matched virgin controls. The observations reported here are generally in accordance with the above findings in that a suppressive influence against nascent tumour development is detectable in these animals following pregnancy. Although these experiments do not demonstrate that an immunological mechanism was responsible for the tumour suppression observed, our previous observations [7], and those of a number of other investigators [9, 10, 14, 15], provide some support for such a possibility.

There is now evidence that foetal antigens can immunize the mother across the placental barrier [16, 17] but that mechanisms have evolved to suppress the maternal immune response against the foetus [14, 17]. The possibility exists that pre-existing sensitivity to oncofoetal antigens could retard to some extent the outgrowth of small tumour nodules so that at the time of development of antitumour activity aroused by the tumour-specific-antigens they have not yet reached a certain critical size and thus susceptible to rejection. This latter idea, embodied in the "sneaking through" concept, was first described by Humphreys *et al.* [18] and subsequently confirmed by Old *et al.* [19] in syngeneic tumour systems. It is possible that a bipartite mechanism as suggested above could be responsible for the suppression of some of the tumours following pregnancy or pre-immunization against foetal tissues. The more strongly immunogenic tumours would thus be the ones most susceptible to rejection under these conditions.

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