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Restoration of the Primary Immune Response and Prevention of Wasting by Pregnancy in Neonatally Thymectomized Female Rats

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Summary

Pregnancy restored the impaired immune capacity of neonatally thymectomized rats towards normalcy, as measured by the serum haemolysin response to sheep red blood cells (SRBC). Pregnancy also prevented the development of the wasting syndrome in these animals. The beneficial effect of one pregnancy lasted at least five months.

In 1965 *Osoba* (1) reported the interesting observation that the depressed immune reactivity of neonatally thymectomized female mice was restored to normal by pregnancy. In eight out of nine thymectomized mice a normal serum haemagglutinin titer following the administration of SRBC was found after one or more pregnancies, which also gave protection against wasting.

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It was considered of importance to investigate if the phenomenon also exists in other species, because it offers a nice model of experimentation: the same animal can be studied as an immunoincompetent individual and – after one delivery – as an immunocompetent individual, without further treatment.

Therefore it was decided to extend the study to rats using larger numbers of animals, and to try also to evaluate for how long time the beneficial effect of one pregnancy – if it was found – would last.

SRBC was chosen as antigen and rats of the inbred Wistar/Fu strain as experimental animals, as it was constantly found (2) that the primary haemolysin response to SRBC in this strain was significantly decreased following neonatal thymectomy.

Material and Methods

Rats of the Wistar/Fu (W/Fu) strain were used. Female rats were thymectomized or sham-operated as described in an earlier paper (3) within 24 hours after birth. Cooling was used as anaesthesia. The animals were given back to their mothers and their intact brothers. At the age of one month the rats were weaned and separated in cages according to sex. When animals were 3-4 months old half of the operated rats were mated with their brothers, whereas the other half was kept unmated. The mated females were allowed to give birth to one litter. The newborn rats were taken away from the mother on the day of birth.

The experimental material thus consisted of four groups:

- 1) neonatally thymectomized uniparous rats
- 2) neonatally thymectomized virgin rats
- 3) neonatally sham-operated uniparous rats
- 4) neonatally sham-operated virgin rats.

All these rats received on the same day a single injection intraperitoneally of a 0.2 per cent solution of washed SRBC in saline, 1 ml per 100 grammes of body weight. Five days later the rats were bled and their serum haemolysin titers were individually determined and expressed as the negative 2 logarithm to the dilution giving 50 per cent haemolysis in our test system.

Autopsy was performed in all animals. If thymic tissue remnants were found in thymectomized rats, these animals were excluded from the experiment.

Experiment 1 comprised 36 Animals. At the time of antigenic stimulation with SRBC they were 4-5 months old; the uniparous rats had given birth 15-55 days earlier.

Results of experiment 1:

The serum haemolysin titers for each group of rats are listed in Table 1. It appears that the average haemolysin titer was significantly higher in the group of thymectomized uniparous rats (group 1) than the average titer of their comparable thymectomized virgin litter mates (group 2). The increased titer of the former group did not, however, reach the level of the group of sham-operated control rats (group 4). Pregnancy did not increase the primary haemolysin response to SRBC in the group of sham-operated rats (group 3).

Thus pregnancy restored to some extent the impaired immune reactivity of the neonatally thymectomized rats. In order to try to evaluate the duration of the beneficial effect of one pregnancy the next experiment was set up.

Experiment 2

Female rats from 23 litters were operated, treated and grouped in exactly the same way as

described in experiment 1, with only the following difference. Whereas the rats from experiment 1 were subjected to SRBC at the age of 4-5 months (1/2 - 2 months after birth in the case of parous rats), SRBC was administered to the rats in experiment 2 at an age of 8-9 months (4-5 months after birth in the case of parous rats). Experiment 2 comprised 59 animals.

Results of experiment 2:

When the experimental animals were 3-4 months old and were to be mated with their brothers, four rats of the neonatally thymectomized group had died of wasting disease and it was apparent that 15 more thymectomized female rats were suffering from the disease. These animals were killed.

The material hereafter comprised 29 non-wasting thymectomized female rats, an equal number of sham-operated rats was included. When 4-5 months old, 15 neonatally thymectomized and 16 sham-operated rats gave birth. Two of the 15 thymectomized animals had started wasting when about 4 months of age, but their symptoms vanished during their pregnancy. Therefore all 15 thymectomized uniparous rats were in good health up to the end of the experimental period.

In the group of neonatally thymectomized virgin rats (group 2), however, an increasing number of rats developed the wasting syndrome and died after the age of 5 months. When the animals were 8-9 months old and had to be tested for their SRBC antibody response, 11 thymectomized virgin rats had succumbed and only three rats were alive.

Table 1 Average serum haemolysin titers, 5 days after challenge with SRBC, of neonatally thymectomized and sham-operated virgin and uniparous rats, 4-5 months old, and 1/2 - 2 months after delivery. The t test gave statistical significance between 0.02 and 0.05% between group 1 and 2

	Haemolysin titer	SEM
group 1, thymect. uniparous	4.1 ±	1.0
group 2, thymect. virgin	1.3 ±	0.7
group 3, sham-op. uniparous	5.9 ±	1.3
group 4, sham-op. virgin	5.8 ±	1.3

Table 2 Average serum haemolysin titers, 5 days after challenge with SRBC, of neonatally thymectomized and sham-operated virgin and uniparous rats, 8-9 months old and 4-5 months after delivery

	Haemolysin titer	SEM
group 1, thymect. uniparous	5.8 ±	0.5
group 2, thymect. virgin	3.0 ±	1.6
group 3, sham-op. uniparous	7.1 ±	0.1
group 4, sham-op. virgin	6.2 ±	0.2

Table 2 shows the average haemolysin titers of the four groups of animals. It appears that again the group of neonatally thymectomized uniparous rats have titers almost as high as their comparable sham-operated litter mates, whether virgin or uniparous.

It can be concluded from experiment 2, that pregnancy protected neonatally thymectomized rats from wasting disease and increased their immune reactivity as measured by their primary immune response to SRBC over a period of at least five months.

Discussion

The fact that pregnancy reconstituted the immune responsiveness of adult rats thymectomized at birth could be due to either the passage of a humoral immunocompetence-inducing factor or hormone, or to the passage of lymphoid cells from foetus to mother. The former interpretation would be in keeping with the thymic humoral factor concept which has emerged from experiments where neonatally thymectomized mice were protected against wasting and had their capacity to reject allografts and to form humoral antibodies against SRBC restored by the implantation of thymic tissue in cell tight millipore diffusion chambers (4, 5, 6, 7, 8, 9) or by the administration of cell free thymic extracts (10, 11, 12, 13, 14, 15).

The latter interpretation: that lymphoid cells from the foetus, they themselves being in an unresponsive state, should by passage in sufficient numbers to the mother's circulation be able to confer immune reactivity upon her seems unlikely. *Osoba* (1) who also favoured the former possibility furthermore gave some experimental evidence against the latter hypothesis: His neonatally thymectomized female mice were mated to males of another strain. The parous females rejected skin grafts from these males in the same way as other foreign skin grafts; this makes it improbable that the mothers housed and were tolerant for hybrid cells from the foetuses.

In a study of the influence of neonatal thymectomy upon the offspring in mice, *Elders, Parham and Hughs* (16) made the interesting observation that there was no change in organ weights of newborn mice from thymectomized and sham-operated mothers except for the thymus weight which was significantly higher in the offspring of thymectomized mothers. This might indicate the existence of homeostatic interrelationships in thymic function between mother and foetus.

In the preceding years the wasting disease did not develop in response to neonatal thymectomy in W/Fu rats under our housing conditions except for sporadic cases, not even in long-term experiments where virus infection was conferred upon the thymectomized animals (17). The appearance of wasting disease in the majority of untreated thymectomized rats in our second experiment was therefore a new experience. In spite of efforts to trace which changes in the environment caused wasting disease to appear, we were not able to disclose any. Its occurrence reduced the group of neonatally thymectomized virgin rats in experiment 2 to three individuals; the group was thus statistically impaired, and besides one could suspect the surviving three rats to be in an unusually good immunological state. Therefore it was considered relevant not to make statistical analysis including this group.

We do not know for how long time the effect of pregnancy upon the immune functions in thymectomized mice and rats lasts. *Elders et al.* (16) who also observed protection against wasting by pregnancy in mice stated that the "protective factor provided by pregnancy may not persist indefinitely, since 9 or the 10 animals dying spontaneously were pregnant more than one time". In experiment 2 of the present investigation, the immunologic responsiveness of thymectomized rats was still restored to the subnormal values five months after delivery of a litter.

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