TRANSPLACENTAL INFLUENCE OF THE THYMUS

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This report presents evidence for the proposition that the thymus is able to influence the recirculating lymphocyte population in ways other than through the provision of cells. The operation of humoral mechanisms, initiated by the thymus, is inferred from the demonstration that changes in lymphocyte levels consequent upon thymectomy are shown to operate across the placenta.

Following the observation that the immunological responsiveness of neonatally thymectomized mice could be restored if diffusion chambers containing implants of embryonic or neonatal thymic tissue were placed in the peritoneal cavity (1), Osoba (2) demonstrated that the response to sheep erythrocytes and skin allografts of neonatally thymectomized female mice that had born at least one litter resembled that of sham-thymectomized females. Peripheral blood lymphocyte levels in these parous mice remained at the levels characteristic of thymectomized animals. In an extension of this investigation, it was found that pseudopregnancy failed to restore immunological competence and also that removal of the fetuses from the uterus before 14 days of gestation vitiated the restorative effect of pregnancy on immunological reactivity of the mother (3). In contrast to this report, Stutman and Good (4) were unable to detect any restoration of the capacity to induce graft-versus-host reactions on the part of spleen cells from neonatally thymectomized mice that had been pregnant.

Materials and Methods

Rats. Rats of the inbred PvG/c strain were used exclusively in these experiments with the exception of a group of normal DA males which were mated with the thymectomized PvG/c females in section e.

Thymectomy. Thymectomy was performed within 24 h of birth in the experiments reported in sections a to d, but was deferred for 2 days after birth in the case of the rats used in section e. The operation was performed under hypothermic anesthesia. The sternum was split to expose the thymus completely, and its margins were then separated from surrounding tissues by blunt dissection before the use of gentle suction to remove the organ. The wound was sutured and dressed with collodion. Blood for leukocyte counting was collected from the tail vein of rats anesthetized with ether. Differential counts were performed on Wright-stained smears.

Results

Peripheral blood lymphocyte levels were monitored in two basic situations. Sections a and b report the effects of thymectomy performed on newborn rats produced by several generations of thymectomized parents. It was reasoned that this protocol would tend to minimize prenatal influences exerted on the lymphoid system of fetal rats which were to be thymectomized on the day of birth.
TABLE I
Peripheral Blood Lymphocyte Levels in Normal and Thymectomized PvG/c Rats

| Status  | Number of rats | Total wbc/ml ($\times 10^{-6}$) | Lymphocytes/ml ($\times 10^{-6}$) |
|---------|----------------|---------------------------------|----------------------------------|
| Normal  | 8              | 155 ± 13                        | 103 ± 08                         |
| TX1     | 4              | 97 ± 09                         | 43 ± 10                          |
| TX2     | 8              | 118 ± 12                        | 25 ± 08                          |
| TX3     | 8              | 103 ± 24                        | 09 ± 03                          |
| TX4     | 6              | 67 ± 06                         | 08 ± 02                          |

* TX1, neonatally thymectomized progeny of normal parents, TX2, neonatally thymectomized progeny of thymectomized parents, TX3, neonatally thymectomized progeny of thymectomized parents and grandparents, TX4, neonatally thymectomized progeny of thymectomized parents, grandparents, and great grandparents

sections c, d, and e, the impact of pregnancy upon the markedly reduced circulating lymphocyte levels characteristic of the thymectomized progeny of thymectomized breeders was studied.

(a) Survival of the Neonatally Thymectomized Offspring of Thymectomized Parents and Grandparents. After neonatal thymectomy of PvG/c rats born to normal parents, wasting occurred in approximately 10% as has been reported from other laboratories (5, 6). In contrast, only 24 of 170 neonatally thymectomized progeny of thymectomized parents and grandparents attained maturity. Furthermore, a number of these survivors subsequently succumbed to progressive wasting.

(b) Circulating Lymphocyte Levels in Neonatally Thymectomized Offspring of Normal and Thymectomized Rats. Table I compares the blood lymphocyte levels of adult PvG/c rats that had been subjected to neonatal thymectomy with those of normal untreated rats. It will be seen that, whereas lymphocyte levels in the thymectomized progeny of normal parents approximated 40% of normal in accord with earlier reports (5), this figure dropped to 25% if the parents had also been thymectomized and to less than 10% if the grandparents additionally were thymectomized. In the case of that group of rats derived from thymectomized parents, grandparents, and great grandparents, it proved impossible to obtain adult survivors unless thymectomy was deferred until the 2nd day of life.

(c) The Effect of Pregnancy on Circulating Lymphocyte Levels in Third Generation Thymectomized Rats. To determine whether the depression of peripheral blood lymphocyte levels that occurs in the neonatally thymectomized offspring of thymectomized parents and grandparents is modified by pregnancy, six third generation thymectomized PvG/c female rats were mated with their male littermates (Table II). It can be seen that lymphocyte levels generally showed an increase (of from 2-10-fold) if measurements taken within a day or two of parturition are compared with earlier recordings. Furthermore, lymphocyte levels tended to decrease markedly during the weeks after pregnancy but to rise again in response to a second pregnancy. As summarized in the footnote to Table II, no consistent trend in lymphocyte levels was observed in normal female rats in response to pregnancy.

(d) Changes in Circulating Lymphocyte Levels in Third Generation Thymectomized Male Rats. As an indication of the extent to which the variations in blood lymphocyte levels described in the preceding section in the third genera-
The Influence of Pregnancy on Peripheral Blood Lymphocyte Levels of Thymectomized Pvg/c Female Rats Bred From Thymectomized Stock

| Rat | Peripheral blood lymphocytes (× 10^9/ml) | Before mating | After 1st mating | Before remating | After 2nd pregnancy |
|-----|----------------------------------------|---------------|-----------------|-----------------|-------------------|
| 1   |                                       | 2.23          | 2.67            | 2.48            | *                 |
| 2   |                                       | 0.2           | 2.67            | 1.22            | *                 |
| 3   | 0.99                                   | 0.73          | 0.56            | 1               |
| 4   | 0.4                                    | 1.48          | 0.51            | 1.82            |
| 5   | 0.33                                   | 0.8           | 0.43            | 1.54            |
| 6   | 1.39                                   | 2.29          | 2.84            | 2.5             |

Pvg/c females that were derived from neonatally thymectomized parents and grandparents and that had been submitted to thymectomy as neonates were mated with thymectomized male siblings. Blood samples on which the counts after pregnancy were based were collected on the day of delivery or on the following day. Male rats were not returned to the cages with the females until 10-12 days after delivery. Blood samples were collected from the females immediately before remating.

The influence of pregnancy on peripheral blood lymphocyte levels was examined in six previously unmated Pvg/c females. Lymphocyte levels increased in three and decreased in three as a result of their first pregnancy, the mean levels changing from 7.18 ± 1.14 to 7.10 ± 1.03 × 10^9/ml. For comparison, the mean levels of the thymectomized group presented in the table (excluding rat number three) rose from 0.71 ± 0.25 to 1.98 ± 0.37 × 10^9/ml.

* These rats did not become pregnant after remating
† This rat produced a stillborn litter from its first pregnancy. It became pregnant for a second time but, before the completion of this pregnancy, wasted and died.

Discussion

Thymectomy performed in the neonatal period on several successive generations of rats resulted in a much more severe reduction of peripheral blood lymphocyte levels in the third and fourth generations of the experiment than that produced in response to thymectomy of the progeny of normal parents.
TABLE III
Peripheral Blood Lymphocyte Levels of Thymectomized PvG/c Male Rats Bred from Thymectomized Stock

| Rat | Initial Peripheral blood lymphocytes ($\times 10^6$/ml) | 14 wk later Peripheral blood lymphocytes ($\times 10^6$/ml) |
|-----|------------------------------------------------------|--------------------------------------------------------|
| 1   | 2.37                                                 | 1.00                                                   |
| 2   | 2.74                                                 | 1.24                                                   |
| 3   | 1.30                                                 | 2.32                                                   |
| 4   | 0.84                                                 | 0.39                                                   |
| 5   | 0.85                                                 | 0.96                                                   |
| 6   | 1.35                                                 | 0.76                                                   |

These PvG/c males were the offspring of, and were mated with, the female rats in Table II. Blood samples were taken from the males before mating and again, 14 wk later, at the completion of the experiment.

TABLE IV
The Influence of Pregnancy, After Mating With Normal DA Males, on Peripheral Blood Lymphocyte Levels of Thymectomized PvG/c Females Bred From Thymectomized Stock

| Rat | Before mating Peripheral blood lymphocytes ($\times 10^6$/ml) | After pregnancy Peripheral blood lymphocytes ($\times 10^6$/ml) |
|-----|-------------------------------------------------------------|-------------------------------------------------------------|
| 1   | 0.38                                                        | 2.4                                                         |
| 2   | 0.65                                                        | 0.51                                                        |
| 3   | 1.3                                                         | 4.14                                                        |
| 4   | 0.47                                                        | 2.34                                                        |
| 5   | 0.85                                                        | 4.48                                                        |

PvG/c females, the offspring of mating the rats in Tables II and III (that is, their great grandparents, grandparents, and parents had been thymectomized as neonates), were subjected to thymectomy 2 days after birth. When adult, they were mated with normal DA male rats. Blood samples were taken before mating and after delivery of a litter.

Furthermore, the blood lymphocyte count of thymectomized females bred from thymectomized stock was significantly augmented for a period if they became pregnant.

Thymectomy was performed on successive generations of rats in an attempt to eliminate any influence exerted by maternal thymic or lymphoid tissue on the development of the fetal lymphoid system. Ideally, it would be desirable to undertake thymectomy of the progeny of a thymectomized mother at an early stage of fetal development to eliminate the influence of the fetal thymus, but such a procedure would require the use of a larger species of animal. The program of breeding and thymectomy was extended to the third and fourth generations in an attempt to minimize the influence on the fetus of maternal lymphoid tissue which had matured during the fetal life of the mother under the influence of the intact thymus of the mother's own mother. The fact that mean lymphocyte levels did not decrease any further when the fourth generation was compared with the third may indicate that this aim had been achieved. Alternatively, given that the high mortality in third and fourth generations renders the adult survivors a highly selected group, it may merely indicate that a certain minimum lymphocyte level is required for survival to adult life.

Interpretation of decreases or increases in peripheral blood lymphocyte level...
in terms of an animal's lymphocyte complement must be subject to the qualification that interchange between a recirculating and a fixed pool of cells could present in this manner. Nevertheless, the dramatic impact of the sequential thymectomy procedure not only on lymphocyte levels but also on survival of the progeny strongly suggest that an intact maternal lymphoid system is important in the development of the fetal lymphoid system. Whether the humoral products responsible were derived from the maternal thymus itself or from maternal lymphoid tissues which have matured under thymic influence is unknown. It appears probable, however, that humoral products of maternal origin are implicated. The possibility that either or both the lymphopenia and wasting could be attributable to a vertically passaged virus cannot be excluded. It cannot be excluded as an explanation for the phenomena reported in the case of thymectomized progeny of normal parents.

The frequency with which pregnancy effected a marked increase in peripheral blood lymphocyte levels of thymectomized female rats could be simply interpreted as an indication that fetal factors had crossed the placenta to influence maternal lymphoid tissue as readily as this effect occurred in the other direction. Although the capacity of pregnancy and parturition to elevate peripheral blood levels of polymorphonuclear leukocytes has been well documented in humans (7), it is most unlikely that increases in lymphocyte levels of the order of magnitude observed in the present experiments are a normal consequence of parturition. The present observations of normal rats during pregnancy certainly do not reveal such an increase. As regards the mechanism by which lymphocyte levels are increased, the absence of any increase on an occasion when the fetuses were stillborn favors a requirement for a fetal product. The occurrence of an increase after pregnancy as a result of mating with an allogeneic partner excludes the possibility that the fetal product is cellular. However, there is no experimental indication as to whether the putative humoral fetal product is specific for lymphoid tissue or is of a more general hormonal nature. Although it would be simple to postulate that maternal lymphocyte levels were elevated as a result of a factor produced by the fetal thymus, it is not permissible to draw this inference in the absence of experiments in which the thymus was ablated in utero.

Previous reports of the influence of pregnancy on the immunological function of thymectomized females are not in conflict with the present observations. Both Osoba (2) and Stutman and Good (4) deferred examination of immune function until some time after pregnancy, and it is clear from the present experiments that the restorative effects on lymphocyte levels are ephemeral. Thus, Osoba (3) noted that lymphocyte levels at the time of re-examination did not differ significantly from those observed before pregnancy. Although it is not possible to assess the relevance of increases in lymphocyte levels for any restoration of immunological function from the present results, it would not be surprising if they were contributory.

Summary

Peripheral blood lymphocyte levels attained in adult rats that had been thymectomized at birth were markedly reduced if the two or three immediately
preceding generations of rats from which they were bred had been thymecto-
mized as neonates. The capacity of thymectomized animals to survive to adult
life was also drastically reduced if they were bred from thymectomized stock.
Pregnancy as a result of mating with syngeneic or allogeneic males produced an
ephemeral increase in peripheral blood lymphocyte levels of third and fourth
generation thymectomized females. These observations are most readily expli-
cable on the basis of a thymus-derived humoral influence acting directly or
indirectly to influence the circulating lymphocyte population.

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References
1. Osoba, D., and J. F. A. P. Miller. 1963. Evidence for a humoral thymus factor
responsible for the maturation of immunological faculty. Nature (Lond.). 199:653.
2. Osoba, D. 1965. Immune reactivity in mice thymectomized soon after birth: normal
response after pregnancy. Science (Wash. D. C.). 147:298.
3. Osoba, D. 1973. Effect of pregnancy on the restoration of immunological responses in
neonatally thymectomized female mice. In Contemporary Topics in Immunobiology.
Plenum Publishing Corporation, 2:293.
4. Stutman, O., and R. A. Good. 1973. Thymus hormones. In Contemporary Topics in
Immunology. Plenum Publishing Corporation. 2:299.
5. Arnason, B. G., and C. Wennersten. 1963. Role of the thymus in immune reactions in
rats. II. Suppressive effect of thymectomy at birth in reactions of delayed (cellular)
hypersensitivity and the circulating small lymphocyte. J. Exp. Med. 116:177.
6. Pinnas, J. L., and F. W. Fitch. 1966. Immunological competence of thymectomized
rats to several soluble and particulate antigens. Int. Arch. Allergy Appl. Immunol.
30:217.
7. Sturgs, C. C., and F. H. Bethell. 1943. Quantitative and qualitative variations in
normal leukocytes. Physiol. Rev. 23:279.