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10 June 1964

Thymus: Role in Maturation of Fetal Lymphoid Precursors

Abstract: *Liver cells from homozygous embryonic mice were subjected to serial passage through appropriate thymectomized and sham-thymectomized F_1 hybrids. Passage through a nonthymectomized host was required for maturation and/or proliferation of the potential immunologically competent cells in the embryonic liver.*

The work of Miller (*1*, *2*) and others (*3*) has firmly established the importance of the thymus in the development of immunological competence by the newborn rodent and in the recovery of the homograft response by adult mice that have been irradiated. Two hypotheses have been advanced to explain the role played by the thymus in the development and maintenance of immunologic integrity: (i) A cellular hypothesis suggests that the thymus is an essential if not exclusive source of the immunologically competent cells which populate the peripheral lymphoid tissues (*1*, *3*). (ii) A humoral hypothesis states that the potential immunologically competent cells are derived from other tissues but are dependent to a greater or lesser degree upon a thymic hormone for their stimulus to mature or proliferate or both (*4*). These two hypotheses have not been considered mutually exclusive. Direct evidence of cellular migration from (and into) the thymus has been presented in both newborn and adult mice (*5*); however, the functional significance of these observations remains unclear. On the other hand, Osoba and Miller (*6*) and Levey *et al.* (*7*) have presented convincing evidence that the thymus produces a filterable factor which has a significant effect upon the proliferation and function of distal lymphoid tissues. Other recent work (*8*) has shown that mouse fetal liver and perhaps other nonthymic embryonic tissues contain potential immunologically competent cells. It is the purpose of this report to show that, within the context of the experimental model, potential immunologically competent cells derived from mouse fetal liver are dependent upon the thymus for their "functional maturation" or proliferation or both.

The presence of potential immunologically competent cells in fetal liver was demonstrated by a modified parental F_1 hybrid, "graft-versus-host," method. In contrast to adult lymphoid tissue, fetal liver from a homozygous donor produces no significant mortality when injected into a sublethally irradiated F_1 hybrid, one parental strain of which is identical to that of the embryo (*8*). However, if after 60 days the lymphoid tissues of this primary host are injected into a second F_1 hybrid (one parental strain identical to that of the embryo but the second parental strain differing from the second parent of the primary host) a significant number of deaths occur within 60 days. This may be summarized as follows: *A* fetal liver $\rightarrow$ (*A* $\times$ B) $F_1 \rightarrow$ (*A* $\times$ C) F_1 and, as is the case in these experiments, $\rightarrow$ (*A* $\times$ B) F_1.

Fetal liver was obtained from 13 A/Sn $\times$ A/Sn embryos (20 days old). It was gently suspended in a balanced salt solution and portions were injected into thymectomized or sham-thymectomized adult primary hosts which had just received 500 rad whole body x-radiation. Noninjected, sham-thymectomized, irradiated mice served as controls. After 60 days the survivors from each group were killed individually, the spleen and lymph nodes of each were gently disrupted, and the resultant cell suspension was injected into two sublethally irradiated secondary recipients. After 60 days the surviving secondary hosts were killed, and their lymphoid tissues were transferred as described to sublethally irradiated (500 rad) tertiary hosts which were genetically identical to the primary hosts. Only the primary hosts were thymectomized or sham-thymectomized. Completeness of thymectomy was confirmed upon subsequent autopsy when the animal died or when it was killed. Death of the primary, secondary, or tertiary hosts within 60 days was taken as evidence for the presence of mature immunologically competent parental cells in the inoculum. The mice were housed randomly, six to eight to a cage. The primary and tertiary hosts were adult male and female (A/Sn $\times$ DBA)F_1 mice and the secondary hosts were (A/Sn $\times$ CBA)F_1 mice.

There were no deaths among the primary hosts or their controls (Table 1). Seven deaths occurred among the eight secondary recipients of parental fetal liver cells passed through sham-thymectomized primary hosts. In contrast, the mortality among the secondary recipients of fetal liver cells passed through *thymectomized* primary hosts did not exceed that found in the control group (one out of eight as against two out of nine). Eight of 14 tertiary recipients of fetal liver that had been passed through thymectomized primary hosts died within 60 days, while there were no deaths in the appropriate control group.

These results again substantiate the presence of potential immunologically

Table 1. Sixty-day mortality in primary, secondary, and tertiary recipients of parental fetal liver. Only primary hosts and primary controls underwent thymectomy or sham-thymectomy. All recipients received 500 rad x-radiation (whole body) prior to injection.

Parental fetal liver (A/Sn)	Treatment of primary host	60-day mortality (No./total)		
		Primary (A/Sn $\times$ DBA)F_1	Secondary (A/Sn $\times$ CBA)F_1	Tertiary (A/Sn $\times$ DBA)F_1
+	Thymectomy	0/4	1/8	8/14
+	Sham	0/4	7/8	
−	Sham	0/5	2/9	0/6

competent cells in fetal liver and demonstrate that, within the context of the experiment, they are dependent upon intact thymic function for their maturation or proliferation or both.

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26 June 1964

Thymectomy: Effect on Secondary Disease in Radiation Chimeras

Abstract. *The pattern of secondary mortality in mice after irradiation and hemopoietic cell transplantation was changed by thymectomy of the recipients 3 weeks before irradiation. In isologous and parent-to-F₁ chimeras excess mortality was occurring later than the usual period of secondary disease. In thymectomized heterologous chimeras secondary mortality was decreased and delayed.*

The importance of the thymus for the development of immune competence in rodents has been the subject of numerous studies (*1*). Although the effectiveness of the thymic implants in diffusion chambers has given an indication of their mode of action in thymectomized animals (*2*), the exact mechanism has not been elucidated. The majority of workers initially postulated a direct effect of the thymus on the development of the lymphoid system, but there is evidence that a major part of the atrophy of the lymphatic system, seen after thymectomy, is a terminal consequence rather than the cause of wasting disease. In addition, there is evidence that suggests an autoimmune reactivity as the cause of this syndrome (*3*).

The thymus has been reported as important not only for the primary development of immune competence after birth but also for its recovery after sublethal irradiation or after irradiation with lethal doses that are followed by bone marrow transplantation (*4*). Miller and co-workers described the effects of thymectomy in young adult mice who then received total-body irradiation in the lethal range and isologous bone marrow treatment. These mice show lower leukocyte counts in the peripheral blood, decreased antibody formation against sheep erythrocytes, and decreased homograft reactivity compared to the controls with intact thymus glands.

In homologous and heterologous "radiation chimeras" the interpretation of the effects of thymectomy is complicated by the immunological consequences of the chimeric state. Although there is no complete agreement on the mechanism by which secondary disease develops, the concept of the "graft-versus-host" reaction is generally accepted. One school of thought emphasizes the resulting immunological incompetence and lymphoid deficiency as the most important factor in the syndrome (*5*), whereas another attributes the syndrome to three causative factors which may vary somewhat in relative importance from case to case. These factors are the direct graft-versus-host immune reactivity, the latent radiation damage, and the immunological incompetence versus invading microorganisms (*6*). In my opinion the latter view is the more plausible.

In the present study the effect of prior thymectomy on the frequency of secondary disease in radiation chimeras was examined. If, in the homologous combination, thymectomy results in a similar decrease in maturation of the immune apparatus, as has been demonstrated in isologous chimeras, the graft-versus-host reaction after injection of immature donor cells will be less severe, while lymphoid deficiency will be increased. If the graft-versus-host immune reaction is the major cause of secondary disease in nonthymectomized chimeras, thymectomy may cause a delay in secondary mortality. If, however, the lymphoid deficiency determines survival, thymectomy may increase the late-mortality rate.

Other modifying factors also are considered. It is conceivable that thymectomy of the host might facilitate the "take" of homologous bone marrow by depressing the host's immune reactivity still further than by radiation alone. That would simulate the situation where a higher dose of bone marrow cells had been given, and this in turn has been described as causing in some cases a more severe secondary disease (*7*).

To obviate this possible complication in homologous transplantation, only parent-to-F_1 combinations were tested. In the heterologous combination no effect of the dose of bone marrow cells on the severity of secondary disease has been described; and even the addition of lymphoid cells to the bone marrow mixture (if injected immediately after irradiation) was described as ineffective in this respect (*8*). Fetal liver was used as a source of hemopoietic cells in the experiments with isologous transplantations and in some of the parent-to-F_1 transplantations in an attempt to increase the occurrence of the lymphoid-deficiency type of secondary disease.

Female F_1 mice (CBA/Rij × C57BL/Rij) or male mice of the parent strains were used as hosts in all experiments. Thymectomy was performed at the age of 8 or 9 weeks by a method similar

Table 1. Survival of thymectomized (T) and control (C) mice after irradiation and transplantation with bone marrow (B.M.) or fetal liver (F.L.) cells.

Mice		Percentage surviving on day					
Group	(No.)	30	60	90	120	150	180
		Isologous F.L. (C57BL and F_1 data pooled)					
T	94	96	94	86	77	67	60*
C	64	100	100	100	97	94	91
		C57BL F.L. → F_1					
T	54	94	85	70	59*	48†	37‡
C	72	99	99	99	99	97	97
		CBA B.M. → F_1					
T	83	100	100	80	64*	57†	53†
C	43	100	100	100	100	100	100
		Rat → F_1 mouse B.M.					
T	336	86	53‡	28‡	16		
C	154	74	18	12	8		

Significance of difference from C: * $p < .05$; † $p < .01$ ‡ $p < .001$