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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References and Notes

1. J. F. A. P. Miller, *Lancet* **1961-II**, 748 (1961).
2. ———, *Nature* **195**, 1318 (1962).
3. C. Martinez, J. Kersey, B. W. Papermaster, R. A. Good, *Proc. Soc. Exptl. Biol.* **109**, 193 (1962); B. G. Arnason, B. D. Jankovic, B. H. Waksman, *Nature* **194**, 99 (1962); D. M. V. Parrot, *Transplant. Bull.* **29**, 102 (1962).
4. D. Metcalf, *Brit. J. Cancer* **10**, 442 (1956); *Brit. J. Haematol.* **6**, 324 (1960).
5. J. F. A. P. Miller, *Lancet* **1963-I**, 43 (1963); *Ann. N.Y. Acad. Sci.* **99**, 340 (1962); J. E. Harris and C. E. Ford, *Lancet* **1963-I**, 389 (1963); *Nature* **201**, 884 (1964).
6. D. Osoba and J. F. A. P. Miller, *Nature* **199**, 653 (1963).
7. R. H. Levey, N. Trainin, L. W. Law, *J. Natl. Cancer Inst.* **31**, 199 (1963).
8. M. L. Tyan and L. J. Cole, *Transplantation* **1**, 347 (1963); *ibid.* **2**, 241 (1964).
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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: * $p < .05$; † $p < .01$ ‡ $p < .001$

to that used by Miller (*9*). The effectiveness of the procedure was checked by histological verification of serial sections of the mediastinal contents. In early experiments control mice were subjected to a sham-thymectomy operation. As these animals proved to be indistinguishable from nonoperated controls the method was discontinued in later experiments.

All mice received a single supralethal radiation dose (912 r, 875 rad) from a 250 kv, 30 ma Maxitron x-ray apparatus (focus-mouse distance 58 cm, dose rate 65 rad/min, half value layer 2.1 mm Cu).

Suspensions of bone marrow cells were prepared as described previously (*8*). The donors of the bone marrow were male or female rats (100 to 125 g) of the inbred WAG strain or 8-week-old female mice of the CBA strain. Fetal liver cells were obtained from C57BL or CBA × C57BL F_1 fetuses on the 15th to 17th day of pregnancy. Each irradiated mouse was injected intravenously within 4 hours after irradiation with 2 to 5 million viable nucleated mouse bone marrow or fetal liver cells or with 17.5 to 26.0 million rat bone marrow cells. The mice were housed five to a cage and given unlimited access to a cubed ration and tap water. Suitable cleaning and sterilization procedures for cages and water bottles were strictly observed (*10*). The mice were observed daily. At least once between the 40th and the 90th day blood samples were taken from the nonisologous chimeras to ascertain whether the peripheral blood cells were donor or host type. Animals which showed signs either of incomplete chimerism or of the presence of thymic remnants were eliminated from the experiment (*11*). Since the results of the isologous experiments (with F_1 fetal liver or C57BL fetal liver) were closely comparable, the data were pooled. The results are presented in Table 1. From these data it is evident that in the isologous and in the parent-to-F_1 combinations secondary mortality is virtually absent in the controls, and late mortality is specific for the thymectomized mice. This late mortality is preceded by a wasting disease similar to that observed in neonatally thymectomized mice. The mortality in the isologous chimeras follows a similar pattern to that reported in a comparable study by Duplan and Monnot (*12*). In the heterologous chimeras there is a severe secondary mortality in the controls in the 2nd month after irradiation. In the thymectomized animals the mortality in the same period is much lower. The survival curve for each of four replicate experiments with rat bone marrow (combined in Table 1) shows this delayed mortality in the thymectomized mice.

In conclusion, under the conditions of these experiments thymectomy prior to the establishment of a radiation chimera is associated with a late mortality in those graft-host combinations where the controls show no secondary disease. In the combination in which there appears a severe early secondary disease without thymectomy, prior thymectomy is associated with a decrease and a delay in mortality. Since in the autologous combination a decreased recovery of immune activity in thymectomized chimeras has been described, it seems very likely that a similar disturbance in the immune reactivity of the donor cells will occur in homologous or heterologous chimeras. A decrease in mortality from secondary disease is in complete agreement with the supposition that this severe secondary disease is primarily caused by an immunological graft-versus-host reaction which, like other immune reactions, will be dependent on the presence of an intact thymus for the development of mature immunologically competent cells. Further study seems necessary to analyze the cause of the late mortality in the thymectomized isologous chimeras.

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References and Notes

1. J. F. A. P. Miller, A. M. E. Marshall, R. G. White, *Advan. Immunol.* **2**, 111 (1962); C. Martinez, J. Kersey, B. W. Papermaster, R. A. Good, *Proc. Soc. Exptl. Biol. Med.* **109**, 193 (1962); A. C. Aisenberg, B. Wilkes, B. H. Waksman, *J. Exptl. Med.* **116**, 759 (1962).
2. D. Osoba and J. F. A. P. Miller, *Nature* **199**, 653 (1963); R. H. Levey, N. Trainin, L. W. Law, *J. Natl. Cancer Inst.* **31**, 199 (1963).
3. M. J. deVries, L. M. van Putten, H. Balner, D. W. van Bekkum, *Rev. Franc. Etudes Clin. Biol.* **9**, 381 (1964).
4. J. F. A. P. Miller, S. M. A. Doak, A. M. Cross, *Proc. Soc. Exptl. Biol. Med.* **112**, 785 (1963); A. Globerson, L. Fiore-Donati, M. A. Feldman, *Exptl. Cell Res.* **28**, 455 (1962).
5. J. F. Loutit and H. S. Micklem, *Brit. J. Exptl. Pathol.* **43**, 77 (1962); J. F. Loutit, *Lancet* **11**, 1106 (1962).
6. D. W. van Bekkum, O. Vos, W. W. H. Weyzen, *J. Natl. Cancer Inst.* **23**, 75 (1959).
7. D. W. van Bekkum, L. M. van Putten, M. J. de Vries, *Ann. N.Y. Acad. Sci.* **99**, 550 (1962).
8. O. Vos, M. J. de Vries, C. Collenteur, D. W. van Bekkum, *J. Natl. Cancer Inst.* **23**, 53 (1959).
9. J. F. A. P. Miller, *Brit. J. Cancer* **14**, 93 (1960).
10. D. van der Waay, W. M. T. Zimmerman, D. W. van Bekkum, *Lab. Anim. Care.* **13**, 46 (1963).
11. M. J. de Vries performed the histological studies and D. van der Waay and Miss A. de Witte the serological typing of cells.
12. J. F. Duplan and P. Monnot, *Compt. Rend. Soc. Biol.* **157**, 1211 (1963).
13. Part of this work was performed under contract with Euratom. We thank D. W. van Bekkum, M. J. de Vries, and H. Balner for the stimulating discussions and Miss. M. van Doorninck and P. Lelieveld for technical assistance.

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Alkaline Phosphatase in Peripheral Nerves

Abstract. *By means of a simultaneous coupling azo dye technique, alkaline phosphatase at the node of Ranvier and the incisures of Schmidt-Lanterman has been identified. The presence of this enzyme at these sites may be of metabolic significance.*

Alkaline phosphatase in peripheral nerves has been studied (*1*). Although it has been described in the endothelial walls of the capillaries, its presence in neural tissue has been inconsistent and no mention has been made of its presence at the node of Ranvier nor at the incisures of Schmidt-Lanterman. We have now identified this phosphatase at the above-mentioned node and incisures.

Adult rats and cats were used. The sciatic nerve was removed under nembutal anesthesia. Care was taken to avoid crushing the nerves which upon excision were immediately immersed in cold (4°C) gum arabic for 10 to 20 minutes before the tissue was frozen rapidly in isopentane at −70°C. The period of immersion in gum arabic is varied in accordance with the diameter of the nerve, but it should not exceed 10 minutes for small nerves and 20 minutes for larger ones. The use of this cold hypertonic solution preserves the structure, although good results can be obtained by freezing directly. Next, sections (8 μ) are cu on the Cryostat and allowed to dry for 10 minutes; they are then fixed in cold formol-calcium solution at 4°C for 1 hour, washed for 30 minutes, and transferred to distilled water for 30 seconds. The sections are then placed in the naphthol AS-AN phosphate substrate with Fast red-violet LB (*2*) (*p*H 8.30) at room temperature for 1 hour (*3*). That the substrate have a *p*H of 8.30 is essential for demonstrating the activity at the incisures and nodes, as the capillaries stain over a *p*H range of 7 to 9. After incubation in the substrate, the