

Response to Stressors of Mice Undergoing Graft-versus-Host Reaction

The lymphoid hypoplasia due to graft-versus-host (GVH) reaction leads, among other things, to a decrease in the absolute number of circulating lymphocytes in the peripheral blood^{1,2}. In the present experiments GVH reaction was induced and the response of these lymphopenic mice to different stressors was studied.

Materials and methods. (C57B1 × A) F₁ hybrid mice were used.

Experiment 1. 6–8-week-old mice were injected i.v. each with 50×10^6 homologue spleen cells from parental (A) donors (GVH group). **Experiment 2.** 3-week-old mice were injected i.p. each with 100×10^6 spleen cells from parental (C57B1) donors (GVH group). Controls of matching age were injected with equivalent quantities of hybrid, isologue spleen cells (Control groups).

The establishment of GVH reaction was confirmed by the decrease in absolute lymphocyte count, loss of weight and higher value of the spleen index³. In experiment 1 cold stress was applied by exposing the mice for 4 h to a temperature of 4°C. In experiment 2 the stress was produced by agitating the mice for 2 h in a shaking machine operated at a frequency of 200/min with an amplitude of 25 mm. Lymphopenic reaction was followed up by absolute lymphocyte count taken on blood sampled from the caudal vein immediately before and 4 h after the exposure to stress under standardized conditions.

Results and discussion. **Experiment 1.** In the 4th week after the injection of spleen cells, definite lymphopenia was observed in the animals of the GVH group, though from the characteristic symptoms of wasting only loss of weight could be observed by this time (Figure 1). On the 28th day after injection, 12 mice of GVH group and 10 mice of control group were exposed to cold. The mice of the control group responded with the normal lymphopenic reaction, the absolute lymphocyte count showed a decrease of 80%, on average. The very low values of the absolute lymphocyte count obtained in the GVH group both before and after the exposure to cold did not show any significant change of the average value as a result of cold stress (Figure 2).

Two animals died in the GVH group within a few hours after the exposure to cold. The remaining animals of this group were lost in the 6th–8th week after the injection while exhibiting the characteristic symptoms of runting disease.

Experiment 2. 12 mice of each the GVH and control group were exposed on the 19th day after the injection to the stress of being shaken. By this time the mice of the GVH group suffered from definite lymphopenia (Figure 3), and their average loss of body weight was 4 g. The average body weight in the control group was 18 g, that in the GVH group 14 g.

In the control group the normal lymphopenic reaction with a decrease of 50% in the absolute lymphocyte count was observed after agitating, whereas no significant change of the absolute lymphocyte count was apparent in the GVH group (Figure 3). 2 animals of the latter group

died during the first hour of shaking. All the animals subjected to experiment 2 were killed on the 20th day after injection. The average values of the spleen index was found to be 1.8 for the GVH group and 0.98 for the control group.

The present results are consistent with our earlier observations on mice suffering from lymphopenia induced by neonatal thymectomy and by administration of anti-lymphocyte serum^{4,5}. The experimental data suggest that the mice with chronic or transient lymphopenia, induced

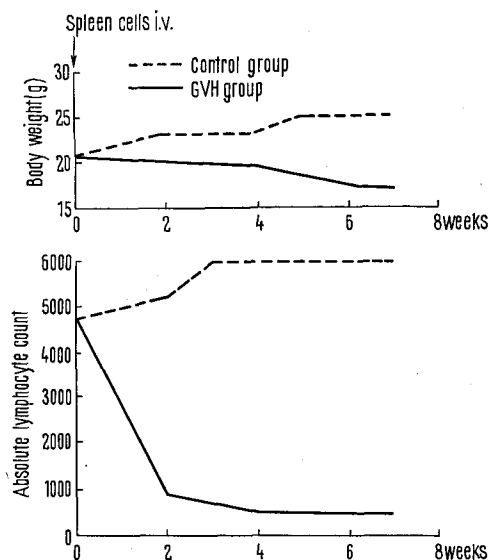


Fig. 1. Average values of weight and absolute lymphocyte count in the animals subjected to experiment 1 after injection with spleen cells.

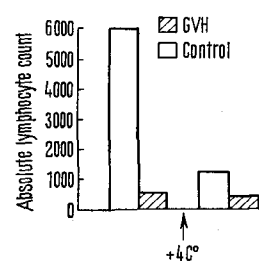


Fig. 2. Average values of absolute lymphocyte count taken in experiment 1 before and after exposure to cold.

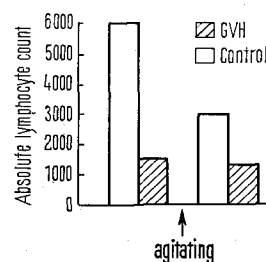


Fig. 3. Average values of absolute lymphocyte count taken in experiment 2 before and after agitating.

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by any method, have lost precisely those lymphocytes which disappear in the lymphopenic reaction, characterizing the state of stress in normal mice. Irrespective of the origin of lymphopenia, the alarm reaction⁶⁻⁸ was anomalous in mice with an absolute lymphocyte count of about 1000, and in addition to the disturbance of immunological adaptation other adaptational disturbances could be observed. It seems that the organism cannot adapt itself appropriately to changed conditions unless the lymphoid system is sound and properly functioning, and the disturbance of the latter is indicated by the anomalous alarm reaction. The present results point to the importance of the lymphoid system in processes other than immunological adaptation.

Zusammenfassung. Nachweis, dass Mäuse nach i.v. oder i.p. Injektion homologer Milzzellen eine Lymphopenie, verbunden mit Gewichtsverlust, entwickeln. Diese Tiere

reagieren nachher auf einen Stress in Form von Abkühlung oder Schütteln nicht mehr mit einem weiteren Lymphozytenabfall.

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⁶ H. SELYE, *Nature* 138, 32 (1936).

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The Effects of X-Irradiation, Applied in Infancy to the Head Region, on the Reproductive System of Female Rats

Recently, we have demonstrated that the whole body exposure of infant female rats to X-rays delays the onset of puberty; this statement was equally applicable to both the animals bearing hypothalamic lesions and to just irradiated ones¹. In this work we intend to describe the reaction of the reproductive tract of female rats to X-irradiation limited to the head region only. The effects of microcoagulation in the hypothalamus in head-irradiated rats are left for a later publication.

Material and methods. The head region of a number of 8-day-old female rats was irradiated with 400, 500, 600, 700 or 800 R of X-rays from a Siemens set (200 kV, 16 mA, filter Cu 0.5 mm, FSD — 34 cm, dose rate 103 R/min) or from a Philips set (200 kV, 16 mA, filter Cu 0.5 mm + Al 1 mm, FSD — 34 cm, dose rate 194 R/min). During the irradiation procedure the animals were anaesthetized with Kemital (thialbarbitone sodium). The number of young per mother was restricted to 6 in all experimental groups and in their controls. A certain number of animals from each of the above groups were mated with normal males.

Results and discussion. In all the experimental groups the opening of the vagina, taken as an index for puberty,

took place at about the same time as in non-irradiated controls. At that time the mean body weight of the experimental animals was slightly below that of the controls (see Table I). As the animals grew older the gain in body weight of the irradiated groups of rats and of the controls proceeded at about the same rate. In connection with this result of ours it is of interest to note that 600 R of X-rays applied to the head only of the neonatal rat produced stunted growth, which could not be repaired by hormone treatment^{2,3}. Stunted growth was also obtained in our strain of random-bred rats when the dose of 600 R of X-rays was applied to the head region of 2-day-old rats⁴.

¹ P. N. MARTINOVITCH, O. K. IVANIŠEVIĆ and J. V. MARTINOVIĆ, *Experientia* 24, 839 (1968) and references cited therein.

² H. D. MOSIER JR. and R. A. JANSON, *Growth* 37, 139 (1967).

³ H. D. MOSIER JR. and R. A. JANSON, *Proc. Soc. exp. Biol. Med.* 128, 23 (1968).

⁴ N. SAVKOVIĆ, J. KAČAKI, R. ANDJUS and K. MALČIĆ, *Strahlentherapie* 130, 432 (1966).

Table I. The occurrence of opening of the vagina and the litter size of 8-day-old head-irradiated rats

Dose of irradiation	Opening of the vagina			Litter size		
	No. of rats	Age (days)	Body weight (g)	No. of rats	No. of litters	No. of new-borns per litter
400 R	22	44.1 ± 5.74	103 ± 9.5	14	14	8.4 ± 2.16
500 R	17	42.8 ± 1.77	101 ± 5.5	4	4	10.2 ± 0.83
600 R	14	43.0 ± 2.90	97 ± 12.5	7	10	8.2 ± 2.32
700 R	17	45.2 ± 6.79	99 ± 11.0	12	15	7.5 ± 2.00
800 R ^a	17	46.2 ± 8.76	100 ± 12.5	6	6	9.0 ± 3.44
Total irradiated	87	44.3 ± 6.01	100 ± 10.6	43	49	8.3 ± 2.45
Non-irradiated	95	44.0 ± 4.57	107 ± 14.0	27	27	8.7 ± 2.01

^a The difference in the mean age at vaginal opening of this group compared with normal controls was not significant at 5% level.