Strain and Substrain Differences in the Recovery of Thymus Function After Whole-Body X-Irradiation of Mice[1] (35077)

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We have previously reported (1) that when sheep red blood cells (SRBC) were given to ICR/Ha mice 24 hr to 6 months after irradiation with 400 or 500 R, hemolysin levels were lower in the thymectomized group than in the sham-thymectomized. When SRBC were given 15 days after irradiation with 300–800 R, hemolysin levels returned to normal in sham-thymectomized mice, but decreased linearly in thymectomized mice as radiation exposure increased.

Several papers have dealt with strain differences in the immune response of mice (2–6). Neonatal thymectomy seems to impair humoral and cellular responses in all strains of mice, as well as in rats, hamsters, and rabbits (7). The recovery of thymus function after radiation injury has been found to be related not only to the strain of the mice but also to the substrain. A linear decrease in hemolysin level with respect to radiation exposure in thymectomized mice was clearly shown in ICR/Ha mice. In other strains, however, even sham-thymectomized mice did not recover immune response in 3 weeks after irradiation.

In the present paper, we are reporting strain and substrain difference in the recovery of the immune response after whole-body irradiation.

Materials and Methods. Male ICR/Ha, C57 BL/6, CBA/St, CBA/H, DBA/2, and BDF_1 (an F_1 hybrid of C57 BL/6 and DBA/2) mice were obtained from our colonies at the Springville Laboratories. Thymectomy and sham-thymectomy were performed as described previously (1).

Thymectomized and sham-thymectomized mice were irradiated 10 days after surgery. Irradiation conditions were as follows: 250 kV, 0.5 mm Cu + 1 mm Al filter, source-to-target distance 50 cm, intensity 60 R/min.

Sheep red blood cells (SRBC) were purchased from the Baltimore Biological Laboratories. The cells were washed thoroughly with saline immediately prior to use, and 0.5 ml of a 20% (vol/vol) suspension was injected intraperitoneally 7 days before the animals were sacrificed.

Sera were obtained from the mice by cardiac puncture. Hemolysin levels were determined by the method of Fahey and Humphrey (8). Serum diluted 10 times with Veronal buffer was used for further serial dilution. Mixtures of 0.1 ml of an 0.5% suspension of washed SRBC with 0.1 ml of diluted serum and 0.1 ml of 15 times diluted guinea pig serum (dried guinea pig serum, obtained from Hyland Laboratories, Los Angeles, California, was reconstituted to the original volume) were incubated for 1 hr at 37° and overnight at 4°. A dilution of serum giving 50% lysis was taken as the end point. Skin grafts were attached with clips devised by Hauschka (9).

Results. Figure 1 shows the relationship between hemolysin levels and the intensity of radiation when thymectomized or sham-thymectomized mice were exposed to radiation (day 0), were injected with SRBC (day 15), and sera were obtained 7 days later. As previously reported (1), hemolysin levels in sham-thymectomized and irradiated ICR/Ha mice recovered to control levels after 22

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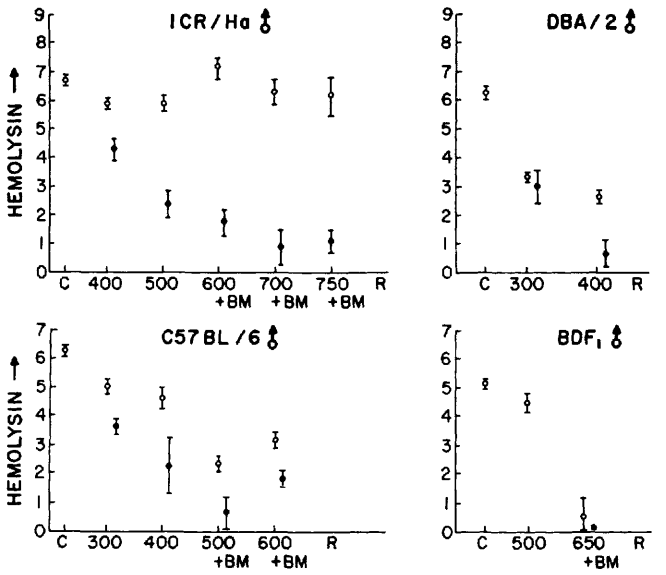

FIG. 1. Relationship between hemolysin levels and various amounts of whole-body X-irradiation. Mice were either thymectomized (solid circles) or sham-thymectomized (open circles) as adults. SRBC were injected intraperitoneally 15 days after X-irradiation, and sera were taken 7 days later.

days, whereas thymectomized and irradiated counterparts generated decreased hemolysin levels with increased radiation exposure. Conversely, even sham-thymectomized and irradiated C57 BL/6, DBA/2, and BDF_1 mice did not recover their antibody-forming ability during the same period. For example, sham-thymectomized and irradiated groups of C57 BL/6 showed quite low hemolysin levels 3 weeks after exposure to more than 500 R. The same phenomenon was observed with DBA/2 at more than 300 R, and with BDF_1 at more than 650 R. In C57 BL/6, 1×10^7 bone-marrow cells were injected 2–4 hr after irradiation with more than 500 R. Sham-thymectomized and irradiated C57 BL/6, however, did not produce high levels of hemolysin, even after the injection of bone-marrow cells. Bone marrow contains precursors of antibody-forming cells (7), but these precursor cells in C57 BL/6 apparently could not differentiate to mature antibody-forming cells. This may indicate that the immunological functions of the thymus do not recover as well in C57 BL/6 (and two other strains) as in ICR/Ha. It is also possible that the difference between ICR/Ha and C57 BL/6 (or two other strains) in hemolysin levels may be due simply to a difference in nonspecific radiosensitivity in these strains.

We found that two substrains of CBA, CBA/St and CBA/H, showed different patterns in recovery of immune competence after exposure to radiation. CBA/St are mice of an inbred strain originated by Dr. L. C. Strong, former director of the Springville Laboratories, and have been maintained at Springville for more than 10 years. CBA/H mice were obtained from Dr. C. E. Ford, of Harwell, England. When CBA/St skin was grafted to CBA/H mice, the interval between grafting and sloughing was 25 ± 6.3 days (mean ± standard error); when CBA/H skin was grafted to CBA/St mice, the interval was 23 ± 3.1 days. These observations suggest that there may be a slight histocompatibility difference between the two substrains. Studies of hematopoietic functions in these two substrans of CBA are to be published elsewhere.

Figure 2 demonstrates the recovery of he-

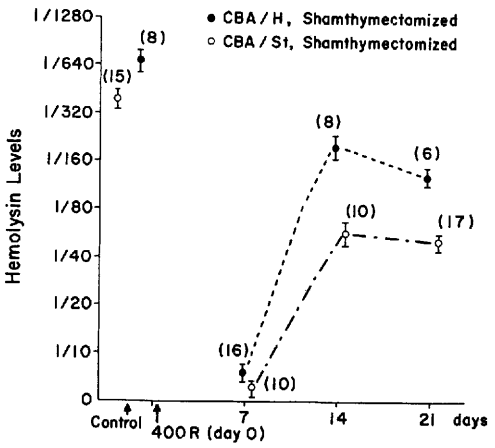

FIG. 2. Hemolysin production in CBA mice after irradiation with 400 R but without injection of bone-marrow cells. The number in parentheses is the number of experimental animals. Mean and standard error are shown at each point.

molysin levels after irradiation with 400 R in these two substrains of CBA. The mice were sham-thymectomized, but no bone-marrow cells were injected. A slight difference in hemolysin levels between CBA/H and CBA/St was noted 7 days after SRBC were injected into these mice. Data obtained through Jerne's plaque tests were similar, and will be published elsewhere.

After sublethal irradiation the recovery of hemolysin levels differed significantly in CBA/H and CBA/St mice (Fig. 2). This difference may be due to a difference in the rate of recovery of thymus functions after sublethal irradiation. To test this possibility, mice of these two substrains of CBA were either thymectomized or sham-thymectomized. After whole-body irradiation, 5×10^6 syngeneic bone-marrow cells were injected intravenously. SRBC were injected at various intervals, and sera were taken 7 days later. If the thymus recovers fastest in CBA/H, significant amounts of hemolysin should be generated in sham-thymectomized CBA/H at the time when neither thymectomized CBA/H nor thymectomized or sham-thymectomized CBA/St produce hemolysin.

Figure 3 indicates the following: (a) In sham-thymectomized and irradiated CBA/H, immune response recovered quite rapidly. (b) In sham-thymectomized and irradiated CBA/St, hemolysin began to appear 35 days

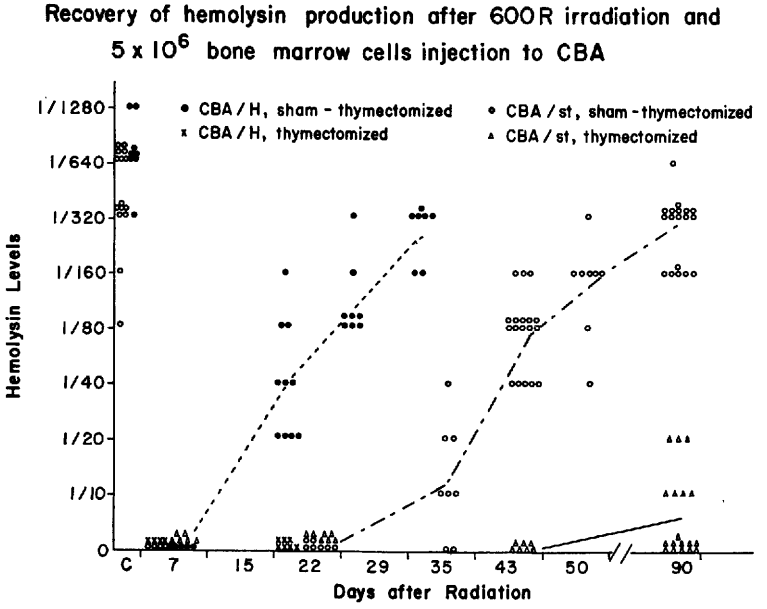

FIG. 3. Hemolysin production in CBA mice after irradiation with 600 R and injection of 5×10^6 syngeneic bone-marrow cells. The abscissa indicates the interval (days) between irradiation and sacrifice.

after irradiation, and gradually increased; 90 days after irradiation, hemolysin levels in CBA/St reached levels equal to those in CBA/H at 35 days. (c) Thymectomized and irradiated CBA/St did not generate hemolysin up to the third month. (d) At day 22, only sham-thymectomized CBA/H generated hemolysin; thymectomized CBA/H and sham-thymectomized or thymectomized CBA /St did not.

Discussion. We have recently shown that the thymus is required for immediate recovery of the immune response after impairment by X-irradiation (1, 10). Although many papers have been published that deal with strain differences in the immune response in mice (2–6), no previous research has been reported that indicates strain differences in the recovery of the immune response after impairment by X-irradiation.

Figure 1 indicates that sham-thymectomized ICR/Ha mice recovered immunocompetence 22 days after various amounts of X-irradiation, and that recovery was independent of the amount of irradiation, provided that the thymus was present.

The difference in hemolysin levels between thymectomized and sham-thymectomized mice is considered to be due to the present or absence of the thymus. Thus, in ICR/Ha, the thymus showed a full recovery of its immunological functions in 22 days after X-irradiation.

In the experiments represented in Fig. 3, 5×10^6 syngeneic bone-marrow cells were injected into lethally irradiated mice. Almost full recovery of hemolysin production was observed in sham-thymectomized CBA/H by day 35 after irradiation, whereas CBA/St were just starting the production of hemolysin at that time. It took 3 months for sham-thymectomized CBA/St to generate amounts of hemolysin equal to those in CBA/H at day 35. Since thymectomized CBA/St produced very low levels of hemolysin up to 3 months after irradiation, hemolysin production in CBA/St earlier than the third month was entirely due to recovery of the irradiated thymus, and thus to the presence of the thymus.

Whereas sham-thymectomized CBA/St did not generate hemolysin at day 22, sham-thymectomized CBA/H demonstrated nearly 50% of normal hemolysin levels. Since no hemolysin was produced in thymectomized mice of either strain, production of hemolysin in CBA/H at that time was due to recovery of the functions of the irradiated thymus. This result indicates that differences in the rate of recovery of functions in the irradiated thymus in mice exist between not only strains but also substrains. From the point of view of the humoral theory of thymus function, it is suggested that the recovery of the production of the humoral thymus factor may differ among different strains or substrains of mice. From the cellular point of view, different strains of mice may exhibit different intervals between irradiation and the production of sufficient numbers of competent thymus cells capable of interaction with cells derived from the bone marrow.

Summary. Adult mice of various strains, either sham-thymectomized or thymectomized, were irradiated. Sheep red blood cells (SRBC) were injected on day 15, and sera were taken on day 22. Sham-thymectomized ICR/Ha produced an almost normal level of hemolysin. C57 BL/6, DBA/2, BDF_1, and CBA, however, could not produce the normal level, but generated decreased amounts of hemolysin even if the thymus was present. There was a difference between the rates of recovery of functions in the irradiated thymus in two substrains of CBA, CBA/St, and CBA/H.

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