Role of the Thymus in the Recovery of Hemolytic Plaque Formation After X-Irradiation[1] (35866)

YUMIKO TAKADA, AKIKAZU TAKADA, AND JULIAN L. AMBRUS

Springville Laboratories, Roswell Park Memorial Institute (New York State Department of Health), Springville, New York 14141; and the State University of New York at Buffalo, Buffalo, New York 14203

We have reported that the recovery of thymus function after X-irradiation in mice was under genetic control (1). Lower hemolysin levels in a substrain of CBA mice (CBA/St) compared with the other substrain (CBA/H) may be due to a difference in either the number of hemolytic plaque-forming cells or in the amount of production of hemolysin by an individual antibody-forming cell. Jerne's (4) plaque test was employed to answer this question.

The possibility of delayed recovery in thymus function in CBA/St mice after X-irradiation may be due to the fact that either the injected marrow cells did not repopulate the thymus and spleen as rapidly as in CBA/H mice, or they migrated into the thymus and spleen of CBA/St mice in smaller number. This question can be answered by injecting chromosomally marked bone-marrow cells (CBA/HT_6T_6) into irradiated CBA/H or CBA/St mice and analyzing the cellular composition of various organs of the hosts at intervals (2, 3). This paper deals with the difference between CBA/H and CBA/St mice in the number of hemolytic plaque-forming cells, thymus and spleen weight, and the extent of repopulation of the bone marrow, thymus, and spleen by CBA/HT_6T_6 marrow cells after X-irradiation and the injection of marrow cells.

Materials and Methods. CBA/H, CBA/St, and CBA/HT_6T_6 mice were obtained from the inbred colonies in the Springville Laboratories. X-Irradiation, thymectomy, and Jerne's plaque tests were performed as described previously (4, 5), and likewise chromosome analysis (2, 3).

Results. Difference between CBA/H and CBA/St in the number of hemolytic plaque-forming cells. Mice both CBA/H and CBA/St, received 400 R of whole-body X-irradiation, and were injected intraperitoneally with sheep red blood cells (SRBC) at certain intervals. Plaque assays were performed 7 days after SRBC injection. Figure 1 shows the results. Controls were unirradiated CBA/H and CBA/St mice. Statistically there is no difference between these two substrains of mice in the number of plaques per control spleen. CBA/H mice showed a larger number of plaques than CBA/St mice following 22 days after irradiation.

Table I shows the results of two experiments: first, thymectomized and sham-thymectomized CBA/H mice were irradiated with 600 R and injected with 5×10^6 bone-marrow cells obtained from CBA/H mice; second, thymectomized and sham-thymectomized CBA/St mice were irradiated with 600 R and injected with 5×10^6 bone-marrow cells obtained from CBA/St mice. These mice were injected intraperitoneally with SRBC 15 or 22 days after X-irradiation. Spleens were removed for plaque assays 7 days after SRBC injection. Thymectomized mice, both CBA/H and CBA/St, showed very small numbers of hemolytic plaques up to 29 days after X-irradiation. Sham-thymectomized CBA/St mice had smaller numbers of plaques than did sham-thymectomized CBA/H mice.

Percentages of chromosome-marked cells (CBA/HT_6T_6) in the bone marrow, spleen,

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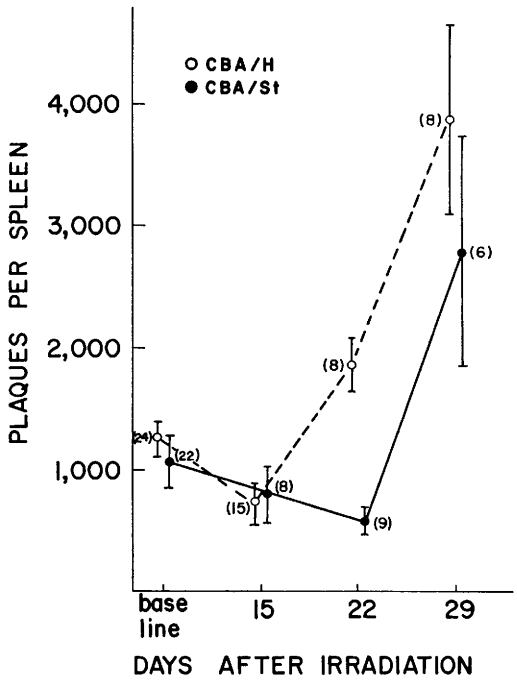

FIG. 1. Recovery of hemolytic plaque formation in CBA/H and CBA/St mice after whole-body irradiation with 400 R. Sheep red blood cells (SRBC) were injected 7 days before mice were sacrificed for plaque assay in the spleens.

and thymus in CBA/H and CBA/St mice. Mice, CBA/H and CBA/St, were irradiated with 600 R, and injected with 5×10^6 CBA/HT_6T_6 bone-marrow cells in 2 to 4 hr. Mice were sacrificed 22 days after X-irradiation, and the distributions of cell types in the marrow, spleen, and thymus were determined.

Table II shows spleen and thymus weights for normal CBA/H and CBA/St mice and CBA/H and CBA/St mice irradiated and injected with CBA/H and CBA/St marrow cells, respectively. Thymus weights for normal mice were a little larger for CBA/St mice than for CBA/H mice, but thymus weights following irradiation with 600 R and injection of bone-marrow cells were larger for CBA/H mice than for CBA/St mice.

Table III shows the results of chromosome analysis. Although there is a slight histocompatibility difference between CBA/St and CBA/HT_6T_6, the percentages of T_6T_6 cells were almost equal in the organs of CBA/St and CBA/H mice. The spleens and thymuses were as fully repopulated with donor marrow cells in CBA/St as in CBA/H mice.

Discussion. We have shown that the recovery of thymus function following X-irradiation is dependent on strains or even substrains of inbred mice (1). CBA/H mice that received 600 R of whole-body irradiation and 5×10^6 bone-marrow cells recovered their antibody-forming ability much more quickly than did CBA/St mice that received the same treatment. Since the same number of bone-marrow cells were injected into each substrain of mice after irradiation, and since thymectomized mice could not produce he-

TABLE I. Plaques per Spleen in Mice Irradiated with 600 R and Injected with 5×10^6 Bone-Marow Cells.

Strain[a]	Thymectomy	Day[b] of SRBC[c] injection	Day[b] of assay	No. of animals	Hemolytic plaques/spleen[d]
H	No	15	22	13	2811 ± 552
St	No	15	22	13	216 ± 86
H	Yes	15	22	16	34 ± 11
St	Yes	15	22	12	12 ± 3
H	No	22	29	19	4510 ± 526
St	No	22	29	8	1130 ± 184
H	Yes	22	29	9	55 ± 26
St	Yes	22	29	16	50 ± 7

[a] Donors and recipients of marrow cells were of the same strain: H = CBA/H; St = CBA/St.

[b] Days after irradiation.

[c] Sheep red blood cells.

[d] Mean ± standard error.

TABLE II. Weights of Spleens and Thymuses of Normal and Irradiated CBA/H and CBA/St Mice.

Organ	Day of sacrifice[a]	CBA/H		CBA/St	
		No. of animals	Wt of organ (mg)[b]	No. of animals	Wt of organ (mg)[b]
Spleen	(Untreated)	19	68.3 ± 2.0	8	69.3 ± 3.5
	22	9	65.8 ± 9.1	9	87.7 ± 5.6
	29	9	70.9 ± 3.6	7	107.3 ± 7.6
Thymus	(Untreated)	19	33.3 ± 1.6	7	41.0 ± 4.5
	22[c]	9	33.6 ± 2.2	9	23.1 ± 2.8
	29[d]	9	41.9 ± 2.6	6	31.0 ± 4.7

[a] Days after whole-body irradiation with 600 R and injection of 5×10^6 marrow cells.

[b] Mean ± standard error.

[c] The mean of the thymus weight on day 22 was shown by the t test to be larger for CBA/H mice than for CBA/St mice at the 95% level of significance.

[d] The difference between the thymus weights for CBA/H and CBA/St mice on day 29 was shown by the t test to be of borderline significance.

molysin even if marrow cells were injected, it was assumed that the difference between these two substrains in the recovery of hemolysin levels was due to difference in the recovery of thymus function.

However, it is possible that the percentage of immunocyte precursor cells among the injected marrow cells was smaller in CBA/St mice than in CBA/H mice. If this was the case, the lower hemolysin levels in CBA/St mice may have been due not to thymus function, but to the smaller number of precursor cells in the marrow cell population. Precursor cells give rise to antibody-forming cells after interaction with thymus-derived cells (6–9) or under the influence of thymic humoral factors (10).

If marrow precursor cells were fewer or

TABLE III. Percentages of CBA/HT_6T_6 Cells in CBA/H and CBA/St Mice Irradiated with 600 R and Injected with 5×10^6 CBA/HT_6T_6 Bone-Marrow Cells.[a]

Organ	CBA/H				CBA/St			
	Animal	Donor	Host	T_6T_6 (%)	Animal	Donor	Host	T_6T_6 (%)
Spleen	1	50	0	100.0	1	50	4	92.6
	2	50	2	96.1	2	50	6	89.3
	3	50	1	98.0	3	50	0	100.0
	4	50	3	94.3				
	5	50	2	96.1				
Thymus	1	50	1	98.0	1	50	3	94.3
	2	50	4	92.6	2	50	6	89.3
	3	50	2	96.1	3	50	5	90.9
	4	50	7	87.7				
	5	50	5	90.9				
Bone marrow	1	50	0	100.0	1	50	7	87.7
	2	50	0	100.0	2	50	2	96.1
	3	50	1	98.0	3	50	2	96.1
	4	50	0	100.0				
	5	50	0	100.0				

[a] Animals were sacrificed 22 days after irradiation and injection.

proliferated less well after antigen administration in CBA/St mice than in CBA/H mice, the number of hemolytic plaques must be smaller in normal CBA/St mice than in normal CBA/H mice. Figure 1 shows no significant difference between these two substrains in the number of plaques at 7 days.

Table II shows that the weight of the thymus was smaller in CBA/St mice than in CBA/H mice after irradiation. This finding may indicate that functional recovery is related to the speed of cellular repopulation of the thymus, since the thymus could "instruct" immigrating cells.

Table III shows that bone marrows, spleens, and thymuses were almost exclusively repopulated with donor-type cells by day 22 after irradiation. These cells, which participated in antibody production, were of donor origin. Unpublished data indicate that almost all cells in the bone marrow, spleen, and thymus were of donor origin in CBA/H or CBA/St mice that had been thymectomized, irradiated with 600 R, and injected with 5×10^6 CBA/HT_6T_6 marrow cells. Data in Table I indicate that CBA/H mice could generate larger numbers of hemolytic plaque-forming cells than CBA/St mice at 22 days after irradiation, and that thymectomized and irradiated CBA mice could not generate hemolytic plaque-forming cells.

Although the weight of the thymus was smaller in CBA/St mice than in CBA/H mice at day 22, it was as fully repopulated with bone-marrow cells in the former as in the latter. Accordingly, it is possible that the smaller number of hemolytic plaques in CBA/St mice following irradiation and injection of marrow cells may be due to the fact that these donor-type marrow cells were not well "instructed" in the thymus, so that they could not participate in antibody formation effectively. In fact, almost all of the dividing cells in the spleens of thymectomized and irradiated mice injected with T_6T_6 marrow cells were of donor origin, but none of them were instructed to react with antigens and with precursors of antibody-forming cells.

On the other hand, some of the donor-type cells in CBA/H mice migrated to the thymus, were instructed, and then emigrated to the spleen, so that they could respond to antigens and precursors. Under the circumstances, recovery of thymus functions following irradiation is influenced by how quickly the thymus is repopulated, how well the ability to instruct is restored, and how quickly the instructed cells seed to peripheral lymphoid organs.

Summary. Relationship between hemolytic plaque formation and repopulation of spleen and thymus after X-irradiation was studied by using two substrains of CBA mice. The CBA/H and CBA/St mice generated almost equal numbers of hemolytic plaques in the spleen at 7 days after intraperitoneal injection of sheep red blood cells (SRBC). The CBA/St mice that received 400 R of whole-body irradiation, or 600 R of irradiation and injection of 5×10^6 marrow cells, generated smaller numbers of plaques at days 22 and 29 than did CBA/H mice that received the same treatment. Bone marrows, thymuses, and spleens of sham-thymectomized and thymectomized CBA/H and CBA/St mice were almost exclusively repopulated with donor-type cells by day 22.

Donor-type cells in the spleens of CBA/H mice had been "instructed" in the thymus, were seeded to the spleen, and responded to antigens, but the spleens of CBA/St mice which were also repopulated with donor marrow cells, had very small numbers of these instructed cells that were capable of responding to antigens.

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1. Takada, Y., Takada, A., and Ambrus, J. L., Proc. Soc. Exp. Biol. Med. **135**, 473 (1970).

2. Takada, A., Takada, Y., and Ambrus, J. L., Proc. Soc. Exp. Biol. Med. **136**, 222 (1971).

3. Takada, A., Takada, Y., Kim, U., and Ambrus, J. L., Radiat. Res. **45**, 522 (1971).

4. Jerne, N. K., Nordin, A. A., and Henry, C., *in* "Cell Bound Antibodies" (B. Amos and H. Koprowski, eds.), p. 109. Wistar Inst. Press, Philadelphia (1963).

5. Takada, A., Takada, Y., and Ambrus, J. L., Radiat. Res. **40**, 341 (1969).

6. Claman, H. N., Chaperon, E. A., and Triplett, R. F., J. Immunol. **97**, 828 (1966).

7. Davies, A. J. S., Leuchars, E., Wallis, J., Marchant, R., and Elliot, E. V., Transplantation **5**, 222 (1967).

8. Miller, J. F. A. P., and Mitchell, G. F., Nature (London) **216**, 659 (1967).

9. Taylor, R. B., Nature (London) **220**, 611 (1968).

10. Miller, J. F. A. P., and Osoba, D., Physiol. Rev. **47**, 437 (1967).

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