The direct demonstration of the proliferation of injected myeloid and lymphoid cells has been done by using the T6 system (1-3). The Harwell group has reported many important findings concerning proliferation of chromosome-marked cells transferred into lethally irradiated mice (4). They have shown that injected marrow cells or lymph nodes/thymus cells did not proliferate in the host thymus until about 2 wk after irradiation and injection of donor cells. They have also shown that donor marrow cells started to appear in the host lymph nodes after day 10, at the time when donor lymph nodes/thymus cells were proliferating in the host lymph nodes.

After the initial experiments by Claman et al. (5), many investigators injected marrow and thymus cells into irradiated hosts together with antigens to study interaction of B and T cells. Although the number of cells injected are varied among different experiments, most people used $1 \times 10^7$ marrow cells and $5-10 \times 10^7$ thymus cells (for example, Claman et al. [6] and Mitchell and Miller [7]). The observations stated above by Micklem et al. (4) were obtained by using $1 \times 10^6$ marrow cells and $1 \times 10^7$ thymus cells.

We have used larger numbers of donor cells for injection and obtained some different findings as to proliferative capacities of donor marrow and thymus cells in irradiated syngeneic host mice.

**Materials and Methods**

Male CBA/H and CBA/HT6T6 mice, 8-9-wk old, were obtained from the inbred mice colonies at the Springville Laboratories. Chromosome analysis was performed according to the methods previously described (2, 8). 1-2 h after whole body irradiation, CBA/H mice were injected with either $1 \times 10^7$ CBA/HT6T6 marrow cells or $1 \times 10^7$ CBA/H marrow cells and $5 \times 10^7$ CBA/HT6T6 thymus cells in the tail vein. These mice were killed at intervals. Smears were made. Slides were stained with Giemsa, and the distribution of the dividing donor and host cells in the bone marrows, spleens, thymuses, and lymph nodes were studied. Mice were irradiated with a General Electric Maxitron 250. Irradiation conditions were as follows: $250 \text{kV}$, $0.5 \text{mm Cu plus 1 mm Al filter}$, at a distance of $50 \text{cm}$ and intensity of $132 \text{R/min}$.

**DISCUSSION AND RESULTS**

Fig. 1 shows the results of experiments in which $1 \times 10^7$ CBA/HT6T6 marrow cells were injected into CBA/H mice irradiated with 800 R. As shown here,
donor marrow cells did not proliferate in the host thymus until day 10, then started to proliferate. The percentages of the dividing marrow cells reached 100% in the thymus on day 30. Donor marrow cells immediately settled and proliferated in the host bone marrow, spleen, and lymph nodes, and comprised 100% of the dividing cell populations in these organs on days 5 and 8. This observation is different from those of Micklem et al. (4), which indicated that donor marrow cells did not proliferate in the host lymph nodes (comprised less than 10%) before day 10, and that less than 50 and 80% of the dividing cells were of donor marrow origin on day 5 in the spleens and marrows, respectively.

Fig. 2 showed the results of experiments in which 1 × 10^7 CBA/H marrow cells and 5 × 10^7 CBA/HT₆T₆ thymus cells were injected into CBA/H mice irradiated with 800 R. As shown here, donor thymus cells did not proliferate in the host bone marrows and spleens even on day 5 or 10, but donor thymus cells comprised more than 80% and about 50% of the dividing cell populations in the lymph nodes and thymus, respectively. The percentages of the donor-type dividing cells gradually decreased to reach less than 5% on day 20 in these organs. This observation is also different from those of Micklem et al. (4), which showed that donor lymph nodes/thymus cells did not proliferate in the host thymuses at any time after X-irradiation and their injection.

We would like to explain these differences in the observations in terms of the different numbers of cells injected. We used large numbers of marrow and thymus cells for injection. These donor marrow cells may have suppressed the division of host marrow and spleen cells due to the feedback regulation of stem cell division, as we proposed before (8-10). 1 × 10^8 marrow cells did not give enough suppressive effects on host cell division, so that many host cells were
Fig. 2. 1 \times 10^7 CBA/H marrow cells and 5 \times 10^7 CBA/HT4T6 thymus cells were injected into CBA/H mice irradiated with 800 R. Five mice were used for each point. •—•, dividing CBA/HT4T6 cells in the host marrows; ▲—▲, dividing CBA/HT4T6 cells in the host spleens; ■—■, dividing CBA/HT4T6 cells in the host lymph nodes; ×—×, dividing CBA/HT4T6 cells in the host thymuses. In order to know the percentages of dividing donor marrow cells in the host bone marrows and spleens, 1 \times 10^7 CBA/HT4T6 marrow cells and 5 \times 10^7 CBA/H thymus cells were injected into CBA/H mice irradiated with 800 R. ○—○, dividing CBA/HT4T6 cells in the host marrows; △—△, dividing CBA/HT4T6 cells in the host spleens. 

dividing in the bone marrow and spleen of the hosts. In the present experiments donor-marrow cells comprised almost always 100% of the dividing cell population in the host marrows and spleens at any time after irradiation of the hosts. On the other hand, donor marrow cells did not proliferate in the host thymus before day 10 even if larger number of marrow cells were injected. We failed to demonstrate dividing donor-type cells in the thymus of the hosts before day 10 even when 3 \times 10^7 marrow cells were injected after 800 R irradiation. The fact that donor thymus cells immediately settled and divided in the host thymus after injection seems to be important. This means that delayed appearance of donor marrow cells in the host thymus was not due to the fact that the thymus did not allow any donor cells to settle after injection, but probably due to the fact that injected donor marrow cells might not have some markers that the host thymus recognized, allowing them to settle and proliferate. Injected marrow cells might have to settle and differentiate in the host bone marrow to acquire membrane markers that were recognized by the thymus. Donor thymus cells apparently had these markers, so that they could settle and proliferate in the thymus immediately after injection. We are currently working on the identification of these markers.

SUMMARY

CBA/HT4T6 bone marrow cells (1 \times 10^7) or CBA/H bone marrow cells (1 \times 10^7) plus CBA/HT4T6 thymus cells (5 \times 10^7) were injected intravenously into lethally (800 R) irradiated CBA/H mice. Chromosome analyses of divid-
ing cells in the host lymphoid and myeloid organs were performed at intervals after irradiation.

Donor marrow cells settled and proliferated in the host bone marrow, spleen, and lymph nodes soon after injection, but donor marrow cells did not proliferate in the host thymus until day 10; then host-type cells were quickly replaced by donor-type cells in the thymus by day 20. On the other hand, donor thymus cells settled and proliferated in the host thymus and lymph nodes soon after injection but they gradually disappeared from these organs. On day 20, a few donor-type dividing cells (of thymus origin) were found in the host lymphoid and myeloid organs.

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