Lymphoid Reconstitution After Transplantation of Congenic Hematopoietic Cells in Busulfan-Treated Mice

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The effects of pretransplant conditioning with high-dose busulfan, a myeloablative but nonimmunosuppressive alkylating agent, on reconstitution of lymphoid tissues by donor cells after bone marrow transplantation (BMT) has not been extensively examined. We used flow cytometric analyses to study the kinetics and extent of lymphocyte reconstitution in C57BL/6 mice (immunophenotype Ly-5.2) given graded doses of busulfan (10 to 100 mg/kg) or total body irradiation (TBI; 900 rad) and hematopoietic cell transplantation (HCT; transplantation of bone marrow and spleen cells) from congenic Ly-5.1 donors. Mice transplanted after 10 mg/kg of busulfan had slow and incomplete lymphoid engraftment, only 6% to 11% of lymphocytes in the peripheral blood, lymph nodes, and spleen were positive for Ly-5.1 at 30 days after transplant, slightly increased to 13% to 20% at 60 days, and stabilized at 40% to 46% by 180 days after HCT. Higher doses of busulfan (20 to 100 mg/kg) provided dose-dependent congenic lymphoid reconstitution. Thirty days after HCT, the range of Ly-5.1 cells in blood, lymph nodes, and spleen of Ly-5.2 recipient mice was 43% to 54% after 20 mg/kg of busulfan, 66% to 71% after 50 to 80 mg/kg, and 77% to 85% after 100 mg/kg. Sixty days after transplant, lymphoid chimera measurement increased to 57% to 68% in 20 mg/kg recipients, 72% to 79% after 35 mg/kg, and 79% to 90% in animals given 50 mg/kg or greater, as seen in radiation chimeras. Despite slower early reconstitution after lower doses of busulfan, donor lymphocytes exceeded 90% to 95% by 120 days after HCT in all mice given at least 20 mg/kg. Even though busulfan lacks directly immunosuppressive properties, virtually complete sustained lymphoid reconstitution by transplanted congenic stem cells occurs after its administration. These observations suggest that pretreatment with busulfan may be effective in gene therapy strategies that involve infusion of autologous marrow cells into which functional genes have been inserted.

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percent of lymphocytes in the peripheral blood, lymph nodes, and spleen were donor-derived (Fig 1). At 60 through 180 days after HCT, the levels of donor Ly-5.1 engraftment in blood, lymph nodes, and spleens of recipients of 10 mg/kg of busulfan were significantly less than values observed in mice transplanted after at least 20 mg/kg (P < .05, two-sample t-test).

In contrast, higher doses of busulfan provided more rapid and complete lymphoid reconstitution by congenic donor cells. In the peripheral blood of Ly-5.2 mice 30 days after HCT following 20 mg/kg of busulfan, greater than 50% of lymphocytes were identified as donor-derived Ly-5.1 cells by flow cytometry. Higher percentages of Ly-5.1 lymphocytes were observed 30 days after HCT in the blood of animals given higher doses of busulfan, ranging from 66% at 35 mg/kg to 85% at 100 mg/kg. The percentage of donor congenic cells in radiation chimera was 82% at 30 days after HCT (Fig 1A). Sixty days after transplant, 68% of peripheral blood lymphocytes were Ly-5.1 in recipients of 20 mg/kg of the drug, 75% to 80% after 35 mg/kg to 80 mg/kg, and 88% after 100 mg/kg of busulfan. Ninety days after HCT, approximately 90% to 95% of peripheral blood lymphocytes were donor-derived in mice given at least 20 mg/kg of busulfan before transplant, similar to that seen in TBI-conditioned recipients, and remained stable at these levels at least as late as 180 days after HCT (Fig 1A).

In the lymph nodes, a substantial percentage of donor Ly-5.1 lymphocytes were detected 30 days after HCT; 43% after 20 mg/kg of busulfan, 57% after 35 mg/kg, 65% to 68% after 50 mg/kg to 80 mg/kg, and 81% after 100 mg/kg (Fig 1B). In TBI-conditioned recipients, 77% of lymphocytes from lymph nodes were donor-derived at 30 days after HCT. Sixty days after transplantation, donor cells accounted for 57% of lymphocytes in lymph nodes of mice conditioned with 20 mg/kg of busulfan, 78% after 35 mg/kg, and 84% to 90% after 50 mg/kg to 100 mg/kg; 90% donor lymphocytes were identified in lymph nodes of radiation chimeras at that time. Three months after HCT, stable congenic lymphoid engraftment was seen in mice given at least 20 mg/kg of busulfan, with 90% to 95% Ly-5.1+ cells in lymph nodes, again similar to that observed after pretreatment conditioning with TBI. Long-term repopulation at these levels of donor lymphocytes was seen as late as 180 days after HCT, with greater percentages of Ly-5.1 cells in animals that received 50 mg/kg or more of busulfan before transplant.

In the spleens of Ly-5.2 mice transplanted 30 days earlier, 54% of lymphoid cells were donor Ly-5.1 in mice given 20 mg/kg of busulfan, 62% to 66% in recipients of 35 mg/kg to 80 mg/kg, and 77% in animals given 100 mg/kg (Fig 1C);
the spleens of TBI-conditioned recipients demonstrated approximately 79% donor lymphocytes. As seen in lymph nodes, there was also a dose-dependent increase in the percentage of Ly-5.1 lymphocytes in spleens of busulfan-conditioned mice; 60 days after HCT, donor cells accounted for 58% of the splenic lymphocytes in mice transplanted after 20 mg/kg of busulfan, 72% after 35 mg/kg, 85% to 90% after 50 mg/kg to 80 mg/kg, and 89% to 90% after either 100 mg/kg of busulfan or TBI. As observed in peripheral blood and lymph nodes, the percentages of donor cells increased to 87% to 90% by 90 days after HCT in animals given at least 20 mg/kg or more of busulfan, compared with 91% in radiation chimeras. Long-term stable repopulation by congenic Ly-5.1 lymphocytes also occurred in spleens of busulfan-conditioned mice; 6 months after transplant, donor splenic lymphocytes accounted for 86% to 87% of the cells in 20 mg/kg or 35 mg/kg recipients, 91% to 93% in mice given 50 mg/kg to 80 mg/kg, and 96% after 100 mg/kg (Fig 1C).

**DISCUSSION**

Busulfan is not classically described as an immunosuppressive agent and cannot be used alone as conditioning for allogeneic BMT. Some morphological studies in rodents given sublethal doses of busulfan without BMT or HCT suggest modest effects of the drug on lymphoid tissues. For example, small lymphocytes in rat marrow decrease to 20%
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