Systemic Monoclonal Antibody Therapy for Eliminating Minimal Residual Leukemia in a Rat Bone Marrow Transplant Model

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In an animal bone marrow transplant (BMT) model that mimics the human clinical condition, we evaluated the effectiveness of monoclonal antibody (MoAb) therapy in eliminating minimal residual disease (MRD) in a leukemic host. Leukemic rats were prepared with marrow ablative but noncurative doses of busulfan (BU) and cyclophosphamide (CY). Two days after syngeneic BMT, rats were treated with MoAb. Although all control rats died of leukemia relapse, 58% of those treated with MoAb were cured without any demonstrable effect on the rate of peripheral blood leukocyte recovery. Furthermore, the level of complement, an important effector in suppressing leukemia proliferation in the normal rat, was not adversely affected by BU/CY, BMT and MoAb. Thus, we demonstrated in an animal model that MoAb therapy may be a useful, nontoxic adjunct to high-dose chemotherapy and BMT in eliminating MRD.

MATERIALS AND METHODS

Animals. Female F1 hybrids of Lewis and Brown-Norway (LBN) rats weighing 150 to 175 g were obtained from Harlan-Sprague Dawley, Walkersville, MD. The animals were housed in shoebox-type plastic cages and received standard laboratory chow and acidified water (pH 2.5) ad libitum. The research protocol was approved by The Johns Hopkins University Animal Care and Use Committee.

MoAbs. The method of MoAb production has been described previously.29 AP64 is an IgM MoAb that recognizes a membrane glycoprotein with a molecular weight (mol wt) of 32 to 33 Kd.30 The MoAb binds to a normal differentiation antigen (cluster designation undetermined) found on human ANLL cells and crossreacts with the homologous antigen found on rat ANLL cells.29 Taking advantage of this ability to bind rat ANLL cells, we evaluated the efficacy and toxicity of MoAb therapy administered to rats with residual disease after marrow ablative, but noncurative doses of chemotherapy and syngeneic bone marrow transplantation (BMT). Such an animal model may help us determine the optimum way in which to use MoAb therapy prior to their evaluation in humans.

Rat leukemia. The rat ANLL used in this study was derived from a female Brown-Norway (BN) rat after treatment with 7,12-dimethylbenzanthracene.31 The leukemia was maintained by serial passage in female LBN rats.

Rat bone marrow preparation. Bone marrow cells were harvested from the femora, tibiae, and humeri of CO2-asphyxiated normal LBN rats. The cells were flushed from the bones with RPMI 1640 (Flow Laboratories, McLean, VA) and made into a single cell suspension by serial passage of the cells through successively smaller bore needles. The cells were washed once and resuspended in RPMI 1640 to a final concentration of 60 x 10^9 nucleated cells/mL.

Minimal residual disease (MRD) model. All rat transplant recipients were kept in laminar airflow hoods (Airclean Engineering, Edgemont, PA) from delivery until 1 month after transplantation. On day 0, the rats were injected with 10^9 BN leukemia cells intravenously (IV). The transplant recipients were prepared with busulfan (BU, gift of Burroughs-Wellcome, Research Triangle Park, NC) 30 mg/kg body weight by gavage on day 13, and cyclophosphamide (CY, gift of Mead-Johnson, Evansville, IN) 100 mg/kg intraperitoneally (IP) on day 14. Twenty-four hours after CY, the rats were rescued with 60 x 10^6 syngeneic nucleated marrow cells to prevent death from marrow aplasia. Forty-eight hours after BMT, the rats received 1 mL of either MoAb AP64-containing ascites (ie, 3.0 mg IgM/kg rat body weight), MoAb...
MH157-containing ascites, NS1-induced ascites or Hanks' Balanced Salt Solution (HBSS, Whittaker M.A. Bioproducts, Walkersville, MD). All rats received medicated, acidified water containing polymyxin, tetracycline, and trimethoprim-sulfamethoxazole for 1 month after BMT. The rats were monitored for leukemia relapse for 120 days after BMT. All 120-day survivors were killed and evaluated for evidence of residual leukemia.

Peripheral blood leukocyte count. Tail vein blood samples were obtained for total leukocyte counts and peripheral blood smears prior to chemotherapy and every other day after BMT for 14 days. The total leukocyte count (ie, the number of leukocytes per microliter) was determined with a Coulter Model Z (Coulter Electronics, Hialeah, FL).

Whole complement (C) assay in the rat. Blood samples (0.5 mL) were collected through the tail vein. Samples were obtained prior to chemotherapy on the day of BMT and daily thereafter for five days. The day-2 samples were obtained immediately before and two hours after MoAb injection. Whole hemolytic C titer was determined using a method previously described.

RESULTS

Evaluation of MoAb in the rat model of MRD. The MRD model was developed to mimic the conditioning regimen used before BMT in human patients. LBN rats were injected with $10^5$ rat ANLL cells on day 0, and treated with BU and CY on days 13 and 14, respectively. Immediately before administration of BU/CY, the rats had an estimated leukemia burden of $10^{11}$ cells, based on the growth kinetics of the BN leukemia in the untreated host. After cytoreductive therapy and BMT, rats demonstrated prolonged survival, but all died of recurrent leukemia by day 76 (mean 63 days, SD ± 12 days). Rats not treated with BU/CY and BMT, However, died by day 25 (mean 20 days, SD ± 4 days), as previously reported.

In this rat model, we evaluated the effectiveness of MoAb AP64 to eliminate MRD existing after BU/CY and BMT. As shown in Fig 1, animals treated with MoAb AP64 had a significant survival advantage over rats treated with MoAb MH157, NS1, or HBSS. Of 24 rats treated with MoAb AP64, 14 (58%) were disease-free at day 120 and two other rats (8%) demonstrated prolonged survival (one died on day 94, and the other died on day 109 after leukemia injection). These long-term survivors died of leukemia relapse limited to the central nervous system (CNS), as demonstrated at autopsy.

Effect of MoAb on hematopoietic recovery. The effect of MoAb AP64 on hematopoietic recovery after BMT is shown in Fig 2. Blood samples were obtained every other day, beginning on the day of BMT to the day of engraftment (defined as the time when the leukocyte count exceeded 1,000 μL and by the presence of granulocytes, reticulocytes and platelets in the peripheral blood). There was no demonstrable difference in the rate of hematopoietic recovery between rats that did and did not receive MoAb therapy. The mean time to recovery was $11 ± 1$ days for rats receiving MoAb AP64 and $11 ± 1$ days for rats receiving HBSS. The incidence of transplant-associated mortality (ie, infections during the aplastic period or other therapy-related toxicities) was not different between rats treated with MoAb AP64 (4%) or the control reagents (8%).
amounts of soluble tumor antigen which can competitively variabilities in the number of residual leukemia cells and the located in sites readily target and the setting of BMT to evaluate MoAb therapy appropriate cytotoxic effector mechanisms. Moreover, such an approach. We found that MoAb administered after these complications in humans is not high.35,36 of occur as a chloroma or reside in the CNS, can Although ANLL ablative therapy and BMT is at a low level. Although we did the amount of MoAb administered may disease than those with a significant tumor burden. Based on these considerations, we selected ANLL as the target and the setting of BMT to evaluate MoAb therapy since leukemia cells are typically located in sites readily accessible to MoAb and the tumor burden after marrow ablative therapy and BMT is at a low level. Although ANLL can occur as a chlora or reside in the CNS, the incidence of these complications in humans is not high.35,36 We evaluated the efficacy of MoAb therapy in a rat transplant model specifically to determine the feasibility of such an approach. We found that MoAb administered after BU/CY and BMT is not only effective in curing many rats with MRD but does so without interfering with the rate or durability of bone marrow engraftment. One-third of the rats, however, still relapsed in the CNS and marrow after MoAb therapy. Leukemia relapse might be accounted for by variabilities in the number of residual leukemia cells and the amounts of soluble tumor antigen which can competitively bind MoAb. These variabilities may be due to several factors (eg, variable plasma levels of BU and CY). In this situation, the amount of MoAb administered may be insufficient for total eradication of ANLL cells in all rats. Although we did not demonstrate any differences in the effectiveness of MoAb therapy despite varying the time of administration (days 17 through 20), the optimum dose and time of administration of MoAb have yet to be determined. In addition, antigenic heterogeneity may account for leukemia relapse in some rats. Although Johnson and Shin39 showed that AP64 bound to a significant percentage of the leukemia cells in all ANLL specimens evaluated, some ANLL cells in any one specimen may lack the antigen recognized by the MoAb. For this reason, we are producing additional crossreactive MoAbs with different antigenic specificities that may be used in combination. In this manner, leukemia cells not binding one MoAb may be recognized by others. The existence of functional effector mechanisms is crucial for the destruction of MoAb-sensitized target cells. Johnson et al34 reported that endogenous C is an important factor in the antileukemia activity of MoAb of the IgM isotype. ANLL suppression was abrogated in rats treated with cobra venom factor in vivo and was partially reversed when C3b was provided on the surface of IgM-sensitized leukemia cells. As shown in Fig 3, total hemolytic C levels were not adversely affected by the cytoreductive therapy and BMT in the rat. The rise in C level observed after the administration of BU/CY may reflect a nonspecific response to generalized tissue injury. Although the contribution of cellular effectors in the aplastic, BU/CY-treated rats has not been fully evaluated, the residual leukemia cells are probably eliminated primarily by the lytic action of complement on IgM-sensitized target cells.

Because of the central role played by C in eliminating the rat leukemia cells, whole C, C3, and C4 levels were evaluated in patients after BU/CY and BMT (data not shown). The total C levels immediately after transplantation were within the normal range in all patients evaluated. Furthermore, no significant alteration in C3 or C4 could be demonstrated. As in the rat model, C levels in human patients are not likely to be a limiting factor in the success or failure of MoAb therapy.

At The Johns Hopkins Oncology Center, patients with ANLL have undergone autologous BMT with marrow purged with 4-hydroperoxycyclophosphamide (4-HC). Although we have observed a significant improvement in overall survival, the actuarial relapse rate still approaches 55%.37,38 Relapse may be the result of (a) inadequate purging of autologous marrow with 4-HC, and/or (b) residual host leukemia after cytoreductive therapy and BMT. Increases in 4-HC and cytoreductive therapy are not possible owing to the unacceptable risks of engraftment failure and extramedullary toxicities, respectively.39,40 MoAb administered systemically may eliminate residual disease in both the purged marrow and the host. Although we have not conducted studies on the effectiveness of MoAb therapy in eradicating human ANLL, we have presented a model that demonstrates the feasibility of such an approach after BMT.

Finally, the property of crossreactivity with rat ANLL cells allows us to examine a MoAb for both efficacy in eliminating MRD and toxicity in vivo in much the same way chemotherapeutic agents have been tested in the past.41 Whether the model will help us predict the appropriate setting for use of MoAb therapy in humans remains to be determined.

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