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Early Vaccination with Tumor Lysate-Pulsed Dendritic Cells after Allogeneic Bone Marrow Transplantation Has Antitumor Effects

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ABSTRACT

Allogeneic bone marrow transplantation (BMT) remains the primary treatment for many hematologic malignancies but has had limited success against solid tumors. The antitumor activity of this treatment approach involves the tumoricidal activity of chemoradiation and the additive graft-versus-tumor activity of donor T cells. However, even with current protocols, some tumors develop resistance and become unresponsive to current therapeutic regimens. To address the problem of resistance and lack of solid tumor activity in allogeneic BMT, we undertook experiments to determine whether the graft-versus-tumor activity of donor T cells could be enhanced in the period immediately after allogeneic BMT with tumor lysate-pulsed dendritic cell (DC) vaccines. Using the B16 melanoma model, we found that the treatment of 6-day tumors with allogeneic BMT and 3 weekly vaccinations of tumor lysate-pulsed DCs starting 3 days after BMT had a significant effect on the growth of murine flank melanomas. This effect was tumor specific and occurred in the absence of full immune reconstitution as measured by donor T cell engraftment and cytotoxic T lymphocyte activity. In addition, DC vaccinations did not appear to exacerbate graft-versus-host disease. These experiments support the feasibility of DC vaccine strategies in the setting of allogeneic BMT.

KEY WORDS

Allogeneic bone marrow transplantation • Dendritic cell vaccines • Immunotherapy • Melanoma

INTRODUCTION

The relapse of hematologic malignancies after bone marrow transplantation (BMT) remains a significant clinical problem because of the frequent lack of response of resistant residual disease to standard chemoradiation protocols [1]. In addition, allogeneic BMT strategies have had only limited success in treating solid tumor malignancies [2-4]. An improvement in clinical outcomes will likely require new and innovative adjuncts to current treatment regimens.

Current intensive chemoradiation protocols are thought to generate most of the antitumor effects seen in autologous BMT [1], whereas the additive graft-versus-tumor (GVT) response mediated by donor T cells is believed to be important in allogeneic BMT [5]. It is thought that the same factors that are responsible for GVT activity also play a role in graft-versus-host-disease (GVHD) [6]. What remains unclear, however, is whether or not the donor T cell populations involved in GVT activity can be expanded in the setting of incomplete immune reconstitution with current immunotherapeutic strategies and whether this can be accomplished without exacerbating GVHD.

The ability to use immunotherapeutic approaches in the post-BMT setting is thought to be dependent on functional immune recovery [7-10] and the exact timing of this process is unclear. Evidence exists, however, that immunization after BMT may be feasible. Effective immunization of children against measles, mumps, and rubella [11] and vaccinations against po-

lio and hepatitis B have been effective when given in the post-transplantation setting [12,13]. In addition, immunization of a human donor with a myeloma-associated paraprotein conferred tumor-specific immunity to a recipient of an allogeneic bone marrow transplant [14]. More recently, several immuntherapeutic strategies in mice have been successful in the setting of allogeneic BMT. In particular, tumor cell vaccines after BMT have been shown to elicit specific antitumor responses without exacerbating GVHD [7,10].

Another immunotherapeutic approach is the use of dendritic cells (DCs). DCs are potent antigen-presenting cells with the ability to stimulate primary and secondary T and B cell responses [15] and have been used as antitumor vaccines in preclinical and clinical studies with encouraging results [16,17]. DCs are thought to be the primary cells that sensitize naive T cells to an initial antigen exposure. Although humoral and cellular effector pathways can contribute to tumor lysis, effector mechanisms involving $CD8^+$ cytotoxic T lymphocytes (CTLs) are thought to be central to the ability to recognize and kill tumor cells [18]. In addition, evidence exists that DCs can directly elicit natural killer cell functions, which can further facilitate tumor destruction [19]. The potency of DC antigen presentation and the subsequent immune response makes it an ideal approach for the generation of an antitumor immune response in early T cell reconstitution.

To investigate whether early DC vaccination strategies can be used in the setting of allogeneic BMT, we examined the ability of early vaccination with tumor lysate (TL)-pulsed DCs soon after BMT to elicit an antitumor effect in mice with established tumor (treatment model). In a tumor protection model, we also tested the ability of early vaccination with TL-pulsed DCs after BMT to elicit protection in naive mice that were subsequently challenged with tumor. Our findings suggest that early vaccination after BMT before full immune reconstitution with TL-pulsed DCs can generate an antitumor effect in animals with established tumor, but an inability to confer protection to naive mice challenged with tumor after TL-pulsed DC vaccination.

METHODS

Mice

Six- to eight-week-old female C57BL/6 (B6, H-2^b, Ly 9.1^-) recipient mice and C.B10-H2b/LilMcdJ (B10, H-2^b, Ly 9.1^+) donor mice were obtained from The Jackson Laboratory (Bar Harbor, Me).The congenic MHC locus of the B10 mice was derived from strain C57BL/10J (H-2^b), whereas most of the remaining loci were of the background strain BALB/cLilMcdJ (H-2^d). Thus, the bone marrow cells derived from the donor B10 mice were matched for major alloantigens, but mismatched for many of the minor alloantigens. C.10-H2b/LilMcdJ (H-2^b) mice do not reject B16 tumor, however; coat color and many other minor alloantigens differ from those in the recipient animal and may play a role in clinical response to vaccines in the setting of BMT. Mice were housed in microisolator cages and fed autoclaved chow and acidified water. The experimental protocol was reviewed and approved by the University Committee on Use and Care of Animals at the University of Michigan.

Media and Cytokines

Complete medium (CM) consisted of RPMI 1640 with 10% heat-inactivated fetal calf serum, 0.1 mM nonessential amino acids, 1 μM sodium pyruvate, 2 mM fresh L-glutamine, 100 U/mL penicillin, 50 μg/mL gentamicin, 0.5 μg/mL fungizone, and 5×10^{-5} M 2-mercaptoethanol. Recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF; specific activity $\geq 5 \times 10^6$ U/mg) was obtained from Immunex Corp (Seattle, Wash) and recombinant murine interleukin (IL)-4 (2.8×10^8 U/mg) was obtained from Schering-Plough Pharmaceutical Research Institute (Kennilworth, NJ). IL-2 was provided by Chiron (Emeryville, Calif) and had a specific activity of 18×10^6 IU/mg protein.

Tumor Cell Lines

B16-BL6 (B16) is derived from B6 mice and is a poorly immunogenic melanoma of spontaneous origin [20]. Tumors were cultured in vivo and used before the fifth passage. For vaccination experiments without bone marrow transplant, 5×10^4 B16 tumor cells (treatment model) and 1×10^5 B16 tumor cells (protection model) were injected subcutaneously into the right flank of B6 recipient mice. For vaccination experiments with bone marrow transplant, 2×10^5 B16 tumor cells (treatment model) and 1×10^5 B16 tumor cells (protection model) were injected subcutaneously into the right flank of B6 recipient mice. Animals with tumors that became ulcerated, caused impaired mobility, or in the judgment of the animal care technicians or principal investigator caused unnecessary stress or discomfort to the animal were euthanized with carbon dioxide narcosis.

Allogeneic BMT

C57BL/6 mice with established 6-day tumor (treatment model) and mice without tumor (protection model) received 10 Gy total body irradiation (TBI; cesium 137 source), split into 2 doses and separated by 3 hours. B10 donor erythrocyte-depleted bone marrow cells, 1×10^7, flushed from the femurs