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Induction of Immunity to Neuroblastoma Early after Syngeneic Hematopoietic Stem Cell Transplantation Using a Novel Mouse Tumor Vaccine

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ABSTRACT

Autologous HSCT has resulted in improved event-free survival in patients with advanced neuroblastoma, but most of these patients still relapse. We previously reported that transient transfection of mouse neuroblastoma cells with plasmid DNA vectors encoding immune costimulatory molecules generates cell-based vaccines capable of inducing potent antitumor T cell immunity. In this study, we explored the effectiveness of tumor vaccine administration soon after HSCT. Soon after transplantation, only vaccinated mice that had received an adoptive transfer of syngeneic T cells survived tumor challenge. Tumor protective immunity in the transplant recipients was dependent on $CD4^+$ and $CD8^+$ T cells, and tumor-reactive T cells in the spleens of vaccinated mice could be detected in IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assays. Our data indicate that the adoptive transfer of T cells was absolutely required for induction of protective immunity by the tumor vaccine. Adoptive transfer of T cells accelerated T cell reconstitution, but it also resulted in increased percentages of $CD4^+CD25^+Foxp3^+$ cells soon after HSCT. Treatment of HSC transplant recipients with an anti-CD25 mAb before tumor vaccination inhibited antitumor immunity and significantly decreased the number of IFN-γ-secreting tumor-specific CD4 T cells. However, physical depletion of $CD25^+$ cells from the adoptively transferred splenocytes appeared to increase the efficacy of tumor vaccination. Collectively, these results demonstrate that anti-neuroblastoma immunity can be induced soon after HSCT using a novel cell-based cancer vaccine. However, sufficient numbers of T cells must be added to the graft to achieve protective antitumor immunity, and depletion of $CD25^+$ T cells from adoptively transferred T cells might provide some additional benefit. These translational studies will aid in our development of post-HSCT vaccines for neuroblastoma.

KEY WORDS

Hematopoietic stem cell transplantation • Tumor vaccine • Plasmid vectors • Immune stimulatory molecules • Adoptive immunotherapy • Regulatory T cells

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in children, accounting for 8% of all childhood cancers [1]. It still has one of the highest death rates of all pediatric cancers and is responsible for approximately 15% of childhood cancer deaths. Despite aggressive treatment, the outcome remains poor for many patients with neuroblastoma. Autologous HSCT has resulted in improved event-free survival for patients with severe disease. However, >50% of patients develop recurrent neuroblastoma from residual disease or from contaminating tumor cells in autologous grafts. Because of the high incidence of relapse, more effective treatments targeting minimal residual disease are required.

It has been demonstrated that neuroblastoma cells express tumor antigens that might be recognized by cytotoxic T cells [2-4]. However, in vivo the tumor may be poorly immunogenic due to an absence of critical immune costimulatory molecules. Neuroblastoma cells that have been genetically modified to ex-

press various immune stimulatory molecules have been employed to induce antitumor immunity in experimental animal models. Because individual immune stimulatory molecules might trigger diverse patterns of cellular activation that contribute differentially to the induction and effector phases of the immune response, combinations of immune stimulatory molecules could provide synergistic or additive effects to tumor immune responses. Our experiments have demonstrated synergistic antitumor effects by combining the costimulatory molecules CD80 and CD86 or CD80 and CD137L (4-1BB ligand) [5,6]. We also showed that high-level transfection of neuroblastoma cells to express a panel of 4 immune costimulatory molecules (CD54, CD80, CD86, and CD137L) transformed the tumor cells into a tumor vaccine capable of stimulating a potent T cell response [7]. This vaccine increased the numbers of detectable tumor-specific splenic CTLs in treated animals, and it induced a more effective antitumor response than did tumor cells expressing only CD80 and CD86.

In this study we explored the effectiveness of tumor vaccine administration soon after high-dose therapy and HSCT because this may be an ideal setting to induce effective tumor immunity due to decreased disease burden [8], altered immune regulation [9], and altered T cell homeostasis [10]. Accelerated lymphoid reconstitution of donor or host origin may overcome inherent defects in T cell signaling [11], defects in APC function including antigen processing and/or presentation, or defective T cell costimulation by APCs [12,13]. Manipulation of the T cell repertoire by immunization during post-HSCT immune reconstitution might skew the T cell repertoire toward particular antigen specificities [14]. Results of animal studies have also suggested that vaccination during homeostatic proliferation could facilitate an immune response to weak self-antigens and enhance T cell-mediated antitumor immunity [15-18].

The immediate post-HSCT period is accompanied by immune deficiency as a result of smaller immune effector cell numbers and impaired lymphocyte function [10]. We hypothesized that efficient antitumor immune responses could be induced soon after HSCT only if syngeneic T cells were adoptively transferred at the time of transplantation. In this study, we show that the combination of high-dose TBI, HSCT, T cell add-back, and early post-transplantation tumor vaccination generates potent anti-neuroblastoma immunity in a manner that could be exploited in future clinical trials.

METHODS

Mice

The following strains of mice (6-8 wk of age) were purchased from Jackson Laboratories (Bar Harbor, Me): A/J, C57BL/6 (B6) ($CD45.2^+$; $Thy1.2^+$), congenic B6.PL-*Thy1a* ($CD45.2^+$; $Thy1.1^+$), and B6-45.1 ($CD45.1^+$; $Thy1.2^+$). The animals were housed in the Medical College of Wisconsin Biomedical Resource Center (Milwaukee, Wis), which has been accredited by the American Association for Accreditation of Laboratory Animal Care.

Tumor Cells

Neuro-2a, a mouse neuroblastoma of strain A origin, was obtained from the American Type Culture Collection (ATCC; Manassas, Va). The tumor cells express MHC class I antigens, but are MHC class II negative. An aggressive subclone, designated AGN2a, was derived through sequential in vivo and in vitro passaging [5]. An MHC class II^+ AGN2a cell line (designated AGN2a-CIITA) was derived by stably transfecting AGN2a with a plasmid expression vector (pcDNA3.1[−]; Invitrogen, Carlsbad, Calif) encoding the MHC class II transactivator (CIITA) gene (provided by Dr Suzanne Ostrand-Rosenberg at The University of Maryland, Baltimore County). SaI, a fibrosarcoma cell line derived from an A/J mouse, was obtained from the ATCC.

Antibodies

The following mAbs, with or without a fluorescent label, were obtained from BD Biosciences (BD Biosciences Pharmingen, San Diego, Calif): anti-CD4 (clones GK1.5 and RM4-5), anti-CD8 (clone 53-6.7), anti-CD16/CD32 (clone 2.4G2), anti-CD25 (clones 7D4 and PC61), anti-CD45.1 (clone A20), anti-CD45.2 (clone 104), anti-CD54 (clone 3E2), anti-4-1BBL (clone TKS-1), anti-CD80 (clone 16-10A1), anti-CD86 (clone 37.51), anti-CD90.2 (Thy1.2; clone 53-2.1), and anti-rat IgG2a (clone RG7/1.30). Control antibodies included purified mouse IgG2b and rat IgG2b. Anti-CD90.1 (Thy1.1, clone HIS51) and anti-Foxp3 (clone FJK-16s) mAbs were obtained from eBioscience (eBioscience, San Diego, Calif).

Hybridomas producing anti-CD25 mAb (clone PC61), anti-CD4 mAb (clone GK1.5), and anti-CD8 mAb (clone 2.43) were obtained from the ATCC. These mAbs were produced in our laboratory using Integra CL 1000 bioreactors (Chur, Switzerland). Anti-Thy1.2-, anti-CD4-, anti-CD8-, and anti-PE-conjugated microbeads used for immunomagnetic cell separation were purchased from Miltenyi Biotec (Miltenyi Biotec, Auburn, Calif).

Flow cytometry was used to analyze gene-modified AGN2a cells for cell surface expression of immune stimulatory molecules as described previously [7]. Antibody-stained cells were analyzed with a Becton Dickinson FACScan flow cytometer, and the resulting data were analyzed using Flow-Jo software (Tree Star, San Carlos, Calif).