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B cell tumor vaccine enhanced by covalent attachment of immunoglobulin to surface proteins on dendritic cells

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Abstract

Protein antigens have been covalently linked randomly to surface proteins on immature dendritic cells (DC). This has been achieved under physiological conditions using a heterobifunctional reagent that couples antigens to free thiol groups expressed on DC surface proteins. This results in a significant increase in the amount of antigen that is bound to DC, and the antigen/membrane protein complexes that are formed are rapidly internalized. DC, loaded covalently with either β-galactosidase (β-gal) or a tumor-associated immunoglobulin (Ig) when injected into mice, induce a β-gal- or Ig-specific T cell response, and a protective anti-tumor immunity for tumors expressing either β-gal or the targeted Ig. This response is shown here to be significantly greater than that which is induced by DC that are loaded with these antigens via the conventional antigen pulse protocol. These results establish a novel, safe, and viable approach of enhancing the effectiveness of DC-based vaccination strategies for B cell lymphoma.

Keywords: Dendritic cells; Immunoglobulin; Cancer vaccines; Antigen loading; T cell activation

Introduction

Immature DC capture antigen via several different pathways including (a) macropinocytosis [1], (b) receptor-mediated endocytosis through either C-type mannose receptors [2–6] or Fc γ receptor types I and II that recognize immune complexes or opsonized particles [7], (c) phagocytosis of apoptotic bodies [8–10], viruses, and bacteria [11,12], and (d) binding and internalization of peptide-loaded heat shock proteins [13,14]. Based on this, several different approaches have been used to load DC with target tumor antigens for vaccination. The most common approaches have been either to load immature DC with soluble proteins ex vivo, so that the DC will take up, process, and present antigenic peptides in the context of MHC, or to load mature DC with discrete MHC binding peptides [15]. Although these approaches are attractive due to their simplicity, the amount of antigen bound to DC and the duration of antigen–DC contact are often not optimal to elicit both a strong and sustained T cell response, both of which are necessary requirements for a tumor vaccine to be clinically effective. Indeed, using such approaches, many potentially important epitopes may not be adequately presented by DC due to either the low binding affinity of peptide epitopes to MHC or inadequate binding of soluble proteins containing such epitopes to DC receptors that mediate antigen internalization and subsequent processing [15]. Further, the loading of soluble antigens to immature DC results primarily in the processing and movement of peptides into the MHC Class II compartment resulting in a minimal MHC Class I loading, thereby not optimizing the activation of CD8 T cells which are believed to play a significant role in tumor immunity.

To overcome the limitations of conventional antigen loading, a number of newer approaches are being developed, including transfection of DC with DNA [16–18] or RNA [19]

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encoding tumor-associated antigens and loading DC with preformed complexes of selected peptides and heat shock proteins [20–23]. In addition antibodies directed against several DC receptors have been used to facilitate the internalization and presentation of Ags. For example, the conjugation of antigens to antibodies that are specific for DEC-205 markedly enhances the targeting of antigen to DEC-205 positive DC [24]. While some of these newer approaches have clearly enhanced antigen presentation resulting in the activation of tumor-specific T cells, these approaches are logistically challenging and expensive, and as in the case of targeting antigen to the DEC-205 receptor, they only exploit the targeting of antigen to a single receptor.

A simple protocol has been designed and tested here to increase the binding to and loading of antigens into DC. By covalently coupling antigens to any proteins that contain one or more free thiol groups, it is possible to target multiple plasma membrane molecules on the surface of DC, thereby increasing the amount of antigen that is bound to DC. We report here that DC loaded with either a surrogate tumor antigen, β-gal or a B cell tumor-associated immunoglobulin via this covalent attachment protocol, provoke an MHC Class I-restricted tumor-specific T cell response and a tumor protective immunity, that is superior to that which is induced by DC that are loaded with antigen using a conventional protocol in which the antigen is not covalently coupled.

Materials and methods

Mice and cell lines

Six- to eight-week-old female BALB/c mice were purchased from Taconic (Germantown, NY) and maintained in the animal facility at the State University of New York at Buffalo (SUNYAB). All procedures were performed according to protocols approved by the SUNYAB Institutional Animal Care and Use Committee (IACUC). The CT26 cell line is an *N*-nitroso-*N*-methylurethane induced BALB/c (H-2^d) undifferentiated colon carcinoma. This tumor grows progressively in BALB/c mice after subcutaneous or intravenous injection [25]. The transfection of this tumor with the bacterial lac-Z gene leads to the expression of β-galactosidase in the tumor cells [25]. This variant of CT26, CT26.CL25 has been established as a progressively growing tumor. The CT26.WT and CT26.CL25 were obtained from Dr. Nicholas Restifo (Surgery Branch in the Division of Clinical Sciences, NCI). CT26.WT and CT26.CL25 were maintained in complete RPMI-10 medium [25]. The hybridoma cell line, 1H6, is the clonal product of the fusion of the immunoglobulin-deficient drug-resistant myeloma cell line P3.X63.Ag8.653 with spleen cells from a BALB/c mouse immunized with α1,3-dextran B1355 from *Leuconostoc mesenteroides* [26]. This cell line was grown in hybridoma-SFM.

Reagents

The heterobifunctional reagent, *N*-succinimidyl-3-(2pyridyldithio) propionate (SPDP) and iodoacetamide were purchased from Sigma (St. Louis, MO). The recombinant murine granulocyte/monocyte colony stimulating factor (GM-CSF) was obtained from Peprotech (Rocky Hill, NJ). LPS and β-galactosidase were obtained from Sigma. CpG 1826 (5′-TCCATGACGTTCCTGACGTT-3′) was synthesized in the Biopolymer facility at Roswell Park Cancer Institute.

Preparation of dendritic cells

Bone marrow cells were isolated from BALB/c mice and cultured to generate DC as previously described [27]. Briefly, bone marrow cells were washed in PBS and resuspended at a concentration of 2×10^5 cells/ml in RPMI-10 containing 200 units/ml rMGM-CSF. 10 ml of the cell suspension was plated in a 100-mm Petri dish (BD Biosciences, Bedford, MA) and incubated at 37°C, 5% CO_2 for 9 days. On day 3, 10 ml of complete medium supplemented with 200 units/ml rmGM-CSF was added to the plate. On days 6 and 8, 10 ml of the medium was removed from each plate and replaced with 10 ml of fresh medium containing 200 units/ml rmGM-CSF. At the end of a 9-day incubation, cells were harvested, counted, and plated in tissue culture plates (BD Biosciences, Bedford, MA) at the same density in RPMI-10 containing 1 μg/ml LPS to mature the DCs. The generation of DC was verified by flow cytometry using antibodies to CD80, CD83, CD86, and MHC Class II (data not shown). After LPS or CPG maturation, DC displayed up-regulation of CD80 and MHC Class II (data not shown). The immature DC were sometimes frozen for future use in RPMI-10 containing 10%DMSO and 200 units/ml rmGM-CSF.

Purification of the B cell tumor-associated immunoglobulin (1H6Ig)

A large quantity of the tumor antigen, 1H6Ig protein, was generated and purified by affinity chromatography of gamma globulin derived from serum and ascites obtained from 1H6/T3 tumor bearing mice using a dextran B1355–sepharose affinity column [28]. In brief, the immunoglobulin fractions, isolated by precipitation at 50% saturated ammonium sulfate, were passed through a dextran B1355–sepharose affinity column and eluted with Glycine, pH2.7 and monitored by spectrophotometer at 280 nm. The purity of the 1H6Ig protein was demonstrated using SDS-PAGE gel electrophoresis.

Chemistry of antigen-coupled dendritic cells (AC/DC)

The key reagent in the coupling reaction is the heterobifunctional reagent *N*-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). The coupling of the antigens to free thiol groups on plasma membrane proteins on the surface of DC proceeds in a simple two-step reaction in aqueous medium under very mild conditions [29,30]. To prevent cross-linking of the tumor antigen prior to its covalent linkage to the free thiol groups on the membrane proteins of dendritic cells, the protein antigens are treated with iodoacetamide to reduce free thiol groups prior to SPDP modification, and the iodoacetamide-treated protein