Dendritic cells loaded with apoptotic antibody-coated tumor cells provide protective immunity against B-cell lymphoma in vivo

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The in vitro priming of tumor-specific T cells by dendritic cells (DCs) phagocytosing killed tumor cells can be augmented in the presence of antitumor monoclonal antibody (mAb). We investigated whether DCs phagocytosing killed lymphoma cells coated with tumor-specific antibody could elicit antitumor immunity in vivo. Irradiated murine 38C13 lymphoma cells were cocultured with bone marrow–derived DCs in the presence or absence of tumor-specific mAb. Mice vaccinated with DCs cocultured with mAb-coated tumor cells were protected from tumor challenge (60% long-term survival), whereas DCs loaded with tumor cells alone were much less effective. The opsonized whole tumor cell–DC vaccine elicited significantly better tumor protection than a traditional lymphoma idiotype (Id) protein vaccine, and in combination with chemotherapy could eradicate preexisting tumor. Moreover, the DC vaccine protected animals from both wild-type and Id-negative variant tumor cells, indicating that Id is not a major target of the induced tumor immunity. Protection was critically dependent upon CD8+ T cells, with lesser contribution by CD4+ T cells.

Introduction

Among human cancers, B-cell lymphomas appear among the most susceptible to immunotherapeutic strategies, because of their high rate of response to monoclonal antibodies (mAbs) targeting the B-cell differentiation antigen CD20 and encouraging results from early phase clinical trials of tumor-specific therapeutic vaccines. The availability of both passive and active immunotherapeutic agents against B-cell lymphomas has made them an important testing ground for the development of clinically effective immunotherapies in humans. The best characterized target for active immunotherapy of B-cell lymphoma is tumor-specific immunoglobulin (idiotype, Id). Immunization of patients with Id protein derived from their own tumors can elicit humoral and T cell–mediated immune responses associated with improvements in survival and tumor burden. Traditional Id vaccines consist of Id protein chemically conjugated to the highly immunogenic carrier protein keyhole limpet hemocyanin (KLH) and injected together with an immunologic adjuvant. Because of their potent antigen-presenting properties, dendritic cells (DCs) have been used to augment lymphoma vaccine effectiveness, and durable tumor regressions have been observed after immunization with Id-loaded DCs. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a DC growth and maturation factor, has also been used as an effective adjuvant in Id-KLH vaccines.

However, despite the elegant nature of the Id vaccine approach, shortcomings of this strategy include the requirement of producing a custom-made protein for each patient and limitation of the antitumor response to a single antigen. In contrast, vaccines using whole tumor cells offer the opportunity to elicit immunity against the entire collection of antigens expressed by the tumor. Pulsed DC vaccination using apoptotic tumor cells or lysates has emerged as a popular strategy for immunization against tumors in a variety of preclinical and human studies. While killed tumor cells in the form of apoptotic bodies or freeze-thaw lysates alone display limited immunogenicity, DCs loaded with these preparations have been found to elicit antitumor immunity in a variety of preclinical models and early clinical trials. Other strategies using DCs to present the full repertoire of tumor antigens expressed by tumor cells include fusion with tumor cells or pulsing with tumor-derived RNA. The goal of these approaches is to achieve processing and presentation of exogenous cell-derived antigenic peptides by professional antigen-presenting cells (ie, “cross-presentation”), thereby evoking a CD8+ T-cell antitumor response.

One attractive strategy for increasing tumor antigen cross-presentation is the targeting of IgG-complexed antigens into DCs via Fcγ receptors. Antigen-antibody complexes internalized via Fcγ receptors at the DC surface efficiently enter both the MHC class I and class II antigen-presentation pathways. Several investigators have recently reported that the uptake of killed, mAb-coated tumor cells by DCs via their Fcγ receptors promotes enhanced processing and presentation of multiple tumor antigens to T cells, thereby offering a strategy for whole tumor cell–DC vaccination. Thus, we carried out an in vivo test of this approach in a syngeneic murine B-cell lymphoma model, and compared it with a traditional Id-KLH lymphoma vaccine. We also sought to ensure that the use of whole tumor cells expressing many normal cellular
antigens would not result in autoimmunity, as previously observed in some tumor vaccine models.35-37

Using a well-characterized lymphoma model in which tumor-specific mAbs were available, we demonstrated that vaccination with DCs loaded in vitro with mAb-coated tumor cells can elicit potent protective antilymphoma immunity in vivo. As an immunogen provided by DCs, mAb-coated tumor cells were superior to untreated apoptotic tumor cells or tumor cell lysates. Importantly, the induced immunity was mediated by T cells, and appeared not to be directed at tumor Id. These in vivo findings suggest a therapeutic lymphoma vaccination strategy with potential for clinical translation.

Methods

Mice and cell lines

Six- to 8-week-old female C3H/Sed/Kam mice were bred and housed at the UCLA Defined Pathogen Colony according to institutional guidelines. The carcinogen-induced B-cell lymphoma 38C13 expressing a clonal IgMκ on its surface has previously been described.38 Subcutaneously administered tumor rapidly metastasizes to spleen, lymph nodes, and bone marrow, within 6 to 9 days, resulting in a systemic tumor burden.39 38C13 as well as 38C13-V2, the idiotype negative variant of 38C13,40,41 were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 50 μM 2-mercaptoethanol (cRPMI). The spontaneously arising, C3H-derived fibrosarcoma AG104A42 (kindly provided by Dr Hans Schreiber, University of Chicago, IL) was also grown in cRPMI. All media and supplements were obtained from Invitrogen (Carlsbad, CA).

Generation and loading of DCs with tumor cell antigens

Bone marrow–derived DCs were prepared as previously described43 with minor modifications. Briefly, bone marrow was flushed from the femurs and tibias of mice and depleted of T and B cells by treatment with rat mAbs followed by panning on plates coated with goat anti–rat Ig Abs (Southern Biotechnology Associates, Birmingham, AL). The mAbs used were GK1.5 (anti-CD4), 53-6-7 (anti-CD8) (both from BD Bioscience, San Diego, CA), and RA3-3A1 (anti-B220) (TIB-146; American Type Culture Collection [ATCC], Manassas, VA). Cells were then plated in 6-well culture plates at 3.5 × 107 cells/mL in cRPMI plus 1000 U/mL recombinant murine GM-CSF (BioSource International, Camarillo, CA) and 1000 U/mL recombinant murine IL-4 (R&D Systems, Minneapolis, MN). Medium was replenished after 4 to 5 days. On day 6, nonadherent and loosely adherent cells were harvested by gentle pipetting and were replated at 5 × 105 cells/mL in 25% conditioned cRPMI media with 75% serum-free AIM V medium (Invitrogen) to minimize exposure to FCS antigens, plus cytokines, and cultured overnight in the presence of media alone or tumor cells (± mAbs) at a ratio of 1 DC:3 tumor cells. 38C13 cells were prepared for DC coculture by resuspension in AIM V at 107/mL, gamma irradiation with 30 Gy (Mark I cesium irradiator; Shepherd and Associates, San Fernando, CA) then incubation for 5 hours at 37°C to promote apoptosis. The apoptotic tumor cells were then incubated for 1 hour on ice with mouse anti-38C13 idiotype mAb (S1C5, IgG2a),44 control mouse IgG2a antibody (UPC-10; Sigma-Aldrich, St Louis, MO), or without antibody. S1C5 binds to tumor-specific determinants on the surface IgM of 38C13 lymphoma cells.44,45 The following day, DCs were harvested, washed, and resuspended in HBSS (Invitrogen) for injection.

Evaluation of tumor cell uptake by DCs

Evaluation of tumor cell uptake by DCs was performed as described previously by Dhodapkar et al.32 Briefly, 38C13 cells were dyed green with PKH 67 (Sigma-Aldrich) before coculture with 38C13 for 0 hours versus overnight at 37°C. Samples were analyzed using a FACScalibur flow cytometer and CELLQuest software (BD Bioscience). DCs phagocytosing tumor cells were visualized as double-positive cells.

Production and modification of Id protein

38C13 Id protein was derived from tumor-myeloma cell hybridoma and coupled 1:1 with KLH (Pierce, Rockford, IL) using glutaraldehyde as previously described.46

Vaccinations

DC immunizations consisted of 105 DCs loaded with antigens as described under “Generation and loading of DCs with tumor cell antigens” and were injected subcutaneously in the inguinal region in 200 μL HBSS twice biweekly, alternating between sides. Idiotype vaccinations consisted of Id-KLH conjugate (50 μg Id) in HBSS subcutaneously twice biweekly. Where indicated, mice received a single dose of 500 ng IL-12 (R&D Systems) subcutaneously in the inguinal region coincident with Id-KLH or 55 ng GM-CSF on the day of vaccination and at the same site for 3 days after vaccination as adjuvants.

Humoral immune response assessments

Nine to 14 days following the last immunization, blood was collected via retro-orbital puncture. Serum anti-Id antibodies were quantitated by enzyme-linked immunosorbent assay (ELISA) as previously described.46 Briefly, 96-well Maxisorp plates (Nunc, Naperville, IL) coated with 38C13 IgM were incubated with serially diluted immune sera. Bound Abs were detected with HRP-conjugated goat anti–mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA) using ABTS substrate (Sigma), and absorbance was determined at 405 nm with a SPECTRAMAX PLUS microplate reader (Molecular Devices, Menlo Park, CA). Affinity-purified S1C5 was used to generate a standard curve. To test for antibodies against nonidiotype surface antigens, 38C13 or 38C13-V2 cells were incubated for 4 hours on ice with 5% sera from mice vaccinated with Id-KLH. DCs loaded with opsonized 38C13 cells, or naive age-matched animals. Bound IgG or IgM was detected with FITC-labeled goat anti–mouse IgG(γ) and PE-labeled goat anti–mouse IgM(μ) antibodies (Southern Biotechnology) using flow cytometry.

Tumor challenge

38C13, 38C13-V2, and AG104A were thawed from common dedicated frozen stocks 3 days before tumor challenge and split the day before. For tumor challenge, cells were washed twice in HBSS and diluted to appropriate concentrations in HBSS. Challenge inocula consisted of 1000 or 5000 38C13 cells, 5000 38C13-V2 cells, or 7.5 × 105 AG104A cells subcutaneously above the base of the tail, as indicated. Treatment of preexisting tumors was performed as previously described.15,47 Mice were first injected with 1000 or 5000 38C13 cells subcutaneously above the base of the tail. Two hours later and again 15 days later, mice were immunized with 105 opsonized 38C13-loaded DCs or 50 μg Id-KLH subcutaneously. Eight days after tumor inoculation, mice received a single dose of 100 mg/kg cyclophosphamide (Mead Johnson, Princeton, NJ) intraperitoneally in sterile saline, and were followed for survival as described.

In vivo depletion of T-cell subsets

Groups of 12 mice were vaccinated with opsonized 38C13-loaded DCs as described above and challenged with tumor 2 weeks later on day 0. On days −6, −5, −4, and 0, and weekly thereafter with respect to tumor challenge, mice were injected intraperitoneally with 200 μg T cell–depleting or control mAbs from ascites as previously described.43 Antibodies used were the CD4+ T cell–depleting mAb GK1.5 (rat IgG2b) (BioExpress, Lebanon, NH), control rat polyclonal Ig (Sigma-Aldrich), CD8+ T cell–depleting mAb HB129 (mouse IgG2a; ATCC), or control mouse IgG2a mAb UPC-10.
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Pad Software, San Diego, CA).
Survival differences among groups of mice were assessed using the
Statistical analysis
flow cytometry to determine the proportion of cells expressing CD19,
90 days after immunization, spleens were removed from groups of 4 mice
to determine the effect of the DC vaccine on the B-cell compartment,
treated with HBSS, or opsonized 38C13-loaded DCs, with or
without depletion of CD8
previously treated with HBSS, or opsonized 38C13-loaded DCs, with or
without depletion of CD8
Vaccination with DCs cocultured with antibody-coated
tumor cells provides specific protection against 38C13
lymphoma cells
Bone marrow–derived DCs phagocytose irradiated
antibody-coated tumor cells
To efficiently introduce the entire set of tumor cell antigens to the
host immune system, we chose to coat apoptotic lymphoma cells
with a tumor-specific mAb prior to coculture with DCs. After
30 Gy γ-irradiation and incubation in serum-free medium for
5 hours at 37°C, 38C13 lymphoma cells underwent apoptosis as
demonstrated by annexin V and propidium iodide staining (data not
shown). To opsonize tumor cells for DC coculture, we used the
tumor-specific anti-38C13 Id mAb S1C5, as mAbs against other
clinically relevant antigens such as murine CD20 were not
available. After irradiation, 38C13 cells were bound specifically by
S1C5, but not by a control mAb (Figure S1A, available on the
Blood website; see the Supplemental Materials link at the top of the
online article) and maintained surface expression of IgM for at least
24 hours in the presence of S1C5 (Figure S1B). To study the
influence of mAbs on the bulk uptake of apoptotic 38C13 cells by
DCs, killed, fluorescent-labeled tumor cells were preincubated with
anti-Id mAb, isotype-matched control mAb, or left untreated, and
then cocultured with day-6 DCs labeled with a different color
fluorochrome (Figure 1). Immediately after initiation of coculture
(0 hours), control mAb had no effect on the uptake of tumor cells
by DCs, while tumor-specific mAb boosted the uptake of tumor
fluorochrome more than 4-fold (Figure 1 top panels). After
overnight culture, DC uptake of specific mAb-coated tumor
cells remained elevated (74%) compared with control mAb-
treated (57.6%) or untreated (56.7%) tumor cells (Figure 1 bottom panels). Uptake of opsonized tumor cells did not result in
further up-regulation of class I and II MHC, CD80, CD86, or
CD54 beyond that measured on DCs cultured with untreated
tumor cells or media alone (data not shown). Thus, in our
system, augmentation of DC maturation was not observed, while
a modest enhancement of tumor cell uptake was detected in the
presence of an opsonizing mAb.
Assessment of autoimmunity
To determine the effect of the DC vaccine on the B-cell compartment,
90 days after immunization, spleens were removed from groups of 4 mice
that had received either opsonized 38C13-loaded DCs and survived
challenge with 38C13, DC vaccine without challenge, or naïve age-
matched control mice. Splenocytes from individual mice were analyzed by
flow cytometry to determine the proportion of cells expressing CD19,
B220/CD45, IgM, or IgG (all Abs from BD Bioscience).
Results
Bone marrow–derived DCs phagocytose irradiated
antibody-coated tumor cells

Figure 1. Phagocytosis of irradiated 38C13 lymphoma cells by DCs. Tumor cells
were labeled with PKH 26, irradiated (30 Gy), and incubated for 30 minutes on ice
with media alone, control mouse IgG2a mAb, or anti-idiotype antibody. The tumor
cells were then cocultured for 0 hours or overnight with DCs prelabeled with PKH 67,
and analyzed via flow cytometry. The percentage of DCs double-positive for both
PKH 26 and PKH 67 is reported in the top right of each panel.

In vivo cytotoxicity assay
The in vivo cytotoxicity assay was adapted from that described by Mueller
et al.40 Briefly, 38C13 cells were incubated with 5-(and-6)-carboxyfluorescein
diacetate, succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) to a final concentration of 2.5 μM. Cells were resuspended to 2 × 10^7/mL
and 2 × 10^7 labeled cells were injected intravenously into groups of 4 mice
previously treated with HBSS, or opsonized 38C13-loaded DCs, with or
without depletion of CD8
prior to coculture with DCs. After 4 hours, mice were killed and spleens harvested, pooled among groups, and analyzed by
flow cytometry to detect CFSE-labeled target cells.

Statistical analysis
Survival differences among groups of mice were assessed using the
Kaplan-Meier method with the log-rank test using Prism software (Graph-
Pad Software, San Diego, CA). P values were considered statistically
significant at P less than .05. B-cell compartment data were charted to
display the mean plus or minus standard deviation of marker-positive cells
for each group. For the splenic compartment and enzyme-linked immuno
spot (ELISPOT) data, groups were compared using the paired, 2-tailed
Student t test, and differences considered statistically significant at P less
than .05.
followed for tumor growth, and killed when tumors reached 1.4 cm in diameter.

HBSS (n/H11005 isotype control antibody (n tumor cells (n/H11005 than immunization with a single defined protein tumor antigen. Antigens in killed whole tumor cells provided stronger antitumor effects (Figure 3). Therefore, a DC-based vaccine incorporating the collection of DCs alone, irradiated tumor cells alone, or Id-KLH protein. Mice were challenged with mAb-coated tumor cells, DCs loaded with untreated tumor cells, DCs loaded with mAb-coated tumor cells, DCs loaded with untreated tumor cells, DCs alone, irradiated tumor cells alone, or Id-KLH protein. Mice were challenged with 5000 38C13 tumor cells subcutaneously on day 0, followed for tumor growth, and killed when tumors reached 1.4 cm in diameter. (B) Groups of 10 mice were vaccinated on days 28 and 14 with DCs loaded with mAb-coated tumor cells, DCs loaded with untreated tumor cells, DCs alone, irradiated tumor cells alone, or Id-KLH protein. Mice were challenged with 5000 38C13 tumor cells subcutaneously on day 0, followed for tumor growth, and killed when tumors reached 1.4 cm in diameter.

Vaccination with DCs loaded with irradiated, mAb-coated tumor cells provides tumor protection superior to that provided by Id-KLH vaccination

To determine the relative efficacy of the mAb-opsonized tumor cell–DC vaccine and a standard lymphoma Id-KLH vaccine, we combined survival data from 7 individual experiments that included both the Id-KLH protein and the DC vaccines (for example, Figure 2A). All had the same vaccination schedule, tumor challenge protocol, and end point criteria. This composite analysis showed that the protection provided by the DC vaccine was superior to that provided by Id-KLH (P = .004; Figure 3). Therefore, a DC-based vaccine incorporating the collection of antigens in killed whole tumor cells provided stronger antitumor effects than immunization with a single defined protein tumor antigen.

Vaccination with DCs loaded with mAb-coated tumor cells can eradicate preexisting tumor in combination with chemotherapy

To test the protective efficacy of the opsonized tumor cell–DC vaccine under more stringent conditions, vaccinations were performed in animals bearing preexisting tumor. Mice were inoculated with 38C13 tumor cells on day 0, followed by a single dose of cyclophosphamide on day 8 to cytoreduce the rapidly growing tumor. Vaccinations with DCs loaded with mAb-opsonized 38C13 or Id-KLH were administered on days 0 and 15 after tumor inoculation (Figure 4). Animals treated with HBSS alone plus chemotherapy all died within 3 weeks, whereas tumor was eradicated from 67% of animals vaccinated with DCs loaded with mAb-coated tumor cells (P < .001 vs HBSS). This level of tumor eradication was also statistically superior to that provided by Id-KLH (25%, P = .015). Thus, under the stringent setting of preexisting lymphoma, the DC vaccine provided robust antitumor efficacy that was once again superior to that provided by Id-KLH.

Protective immunity is not dependent on idiotype expression by tumor cells

To determine whether the protective immunity was dependent on surface Id expression by the tumor cells, we used the Id-negative 38C13 variant V2.40,49 38C13-V2 lacks surface expression of IgM due to a frameshift mutation in the leader sequence of the kappa light chain, and, unlike wild-type, 38C13 is insensitive to treatment with anti-Id mAbs. Mice were vaccinated with either DCs loaded with mAb-coated 38C13 cells or Id-KLH plus IL-12, then challenged with either...
wild-type 38C13 or the Id-negative V2 (Figure 5A). While Id-KLH provided some protection against wild-type 38C13 (25%), it provided no protection against V2 (Figure 5A). The DC vaccine, however, provided equal protection against both wild-type 38C13 (75%) and the Id-negative V2 (66.7%) (Figure 5A). Again, the DC vaccine provided significantly better protection against 38C13 than did Id-KLH (P = .01). Thus, the antilymphoma immunity induced by the mAb-opsonized tumor cell–DC vaccine was not dependent on expression of Id at the tumor cell surface.

To further investigate the mechanism of tumor protection after mAb-opsonized tumor cell–DC vaccination, we first sought to determine whether a humoral antitumor immune response was induced. The 38C13 lymphoma is susceptible to control by the mAb-opsonized tumor cell–DC vaccination, we first sought to determine whether a humoral antitumor immune response was induced by vaccination with DCs loaded with mAb-coated tumor cells. Pooled sera from mice vaccinated with Id-KLH, DC loaded with irradiated, mAb-coated tumor cells, or naive control sera were incubated for 4 hours with wild-type 38C13 (left panels) or idiotype-negative V2 (right panels). Bound antibodies were detected with FITC-labeled anti-IgG (left panels) or anti-IgM (right panels) antibodies (filled histograms) using flow cytometry; lines indicate isotype control staining.

Immune sera contained antibodies against Id-KLH readily stained 38C13 lymphoma cells (positive control, Figure 5B upper left panel), immune sera from DC-vaccinated mice contained no measurable antitumor antibodies (Figure 5B middle left panel). Similarly, using the surface Id/IgM-negative V2 variant cells as targets, we found that immune sera also lacked IgM antibodies against non-Id cell surface antigens (Figure 5B right panels).

CD8+ T cells are the principal mediators of antitumor immunity following mAb-opsonized whole tumor cell–DC vaccination

The lack of detectable antitumor antibodies suggested that T cells might play the dominant role in mediating tumor protection after vaccination. To test this, we performed tumor challenge experiments in mice depleted of either CD4+ or CD8+ T cells. DC vaccination in CD8 T cell–depleted mice did not provide any protection against tumor challenge (Figure 6A). Vaccination of CD4+ T cell–depleted mice provided an intermediate level of protection that was significantly lower than control-depleted mice (P = .047) yet significantly higher than CD8+ T cell–depleted mice (P = .027; Figure 6A). In 3 independent experiments, the protective antitumor response was critically dependent on CD8+ T cells.

To further investigate the in vivo mechanisms of antitumor immunity induced by our DC vaccine, an in vivo cytotoxicity assay...
Vaccination with DCs loaded with antibody-coated B-cell lymphoma cells does not deplete the host splenic B-cell compartment

Given our use of whole lymphoma cells in the DC vaccine, the potential existed for induction of autoimmunity against normal B cell–associated antigens. Importantly, at no time in our studies did we observe signs of illness or autoimmunity (weight loss, wasting, behavioral changes) in animals after vaccination, and all survivors appeared healthy up to 75 days after tumor challenge. Nonetheless, we hypothesized that autoimmunity may manifest as a reduction in the normal B-cell compartment after vaccination and/or tumor challenge. Thus, we studied the splenic B-cell compartment in vaccinated mice, comparing animals that had received the mAb-coated tumor cell–DC vaccine and survived a tumor challenge (8 of 12 mice challenged survived), DC vaccine but no challenge (6 mice), and naive age-matched animals (4 mice). Sixty days after challenge, spleens were removed and the percentage of lymphocytes expressing the B-cell markers CD19, B220/CD45, IgG, or IgM was determined in each individual spleen by flow cytometry. There was no statistically significant difference among the percentages of CD19+ and B220/CD45+ cells in the spleens of the 3 groups (Figure 7), indicating that the overall size of the splenic B-cell compartment was not altered by vaccination or tumor challenge. A modest decrease in IgM+ B cells was seen, however (P = .05), matched by a rise in IgG+ cells in the vaccinated groups (P = .04). This was likely indicative of the priming of naïve B cells during vaccination, with resultant Ig class switching from IgM to IgG. Thus, despite the use of whole tumor cells, which contain a mixture of both tumor-associated and normal B-cell proteins, no measurable tissue-specific autoimmune effects could be detected.

Discussion

The principal objective of this study was to carry out an in vivo test of vaccination with DCs loaded with antibody-coated tumor cells in a fully syngeneic model system in order to validate and extend the promising in vitro results obtained using this approach.31-34 We hypothesized that DCs loaded with mAb-opsonized, killed tumor cells would present tumor antigens to T cells more efficiently than DCs loaded with untreated apoptotic cells or tumor cell lysates, and thereby provide superior antitumor immunity in vivo. As observed in several previous in vitro studies,32,33 we found that the addition of antitumor mAb to cocultures of killed tumor cells and DCs resulted in only modestly increased cellular antigen uptake (Figure 1), and was not accompanied by DC maturation. However, pretreatment of the tumor cells with mAb before coculture with DCs resulted in greater antitumor immunity in vivo (Figure 2). Antitumor effects were more marked using mAb-opsonized tumor for DC pulsing in the settings of both tumor challenge (Figure 2) and preexisting tumor (Figure 4). Thus, the enhanced in vitro tumor immunity achieved previously using DCs loaded with mAb-opsonized tumor cells was borne out in our in vivo model.

A second major objective of this study was to compare the efficacy of a mAb-opsonized whole tumor cell–DC vaccine with a traditional lymphoma vaccine (tumor-specific Id protein), and to determine whether this vaccine could elicit immunity against tumor antigens beyond Id. The opsonized whole tumor–DC vaccine was found to offer significantly better tumor protection than Id-KLH vaccination in the prophylactic setting (Figure 3). Importantly, in the more stringent setting of preexisting tumor, the DC vaccine was also more potent than Id-KLH (Figure 4). In these comparative studies, IL-12 and GM-CSF were each used as cytokine adjuvants in some experiments in attempt to achieve improved protection using Id-KLH, yet mAb-opsonized tumor cell–DC vaccines still offered superior tumor immunity in vivo. In 2 independent experiments, we found no survival advantage with GM-CSF as an adjuvant over 38C13 Id-KLH alone (data not shown), and thus in subsequent experiments we used Id-KLH alone or with IL-12.

One explanation for the greater efficacy of the DC–whole tumor cell vaccine may be the more efficient induction of CD8+ T cells recognizing one or more non-Id lymphoma antigens. In the 38C13 model,
antibodies play a major role in mediating tumor protection using Id vaccines, even when Id is delivered by DCs. In contrast, the mAb-opsonized 38C13-DC vaccine did not induce detectable antibodies to Id or other cell-surface antigens (Figure 5B). Using both in vivo depletion studies and an in vivo cytotoxic T lymphocyte (CTL) assay, we found that tumor immunity after mAb-opsonized tumor cell–DC vaccination was critically dependent on CD8+ T cells (Figure 6A,B). To determine whether Id-derived class I MHC–binding epitopes could be the targets of these CTLs, we challenged vaccinated mice with the 38C13 Id-negative variant cell line V2, which does not express kappa light chain. Since the DC vaccine provided equal protection to this cell and wild-type 38C13, kappa light chain–derived determinants can be excluded as possible targets of the induced CTLs (Figure 5A). Yet as V2 still expresses the IgM heavy chain, we cannot exclude heavy chain–derived epitopes as targets of the induced CTLs. However, the 38C13 heavy chain lacks predicted H-2d class I–binding epitopes, and immunization with Id-pulsed DCs, Id-encoding plasmid DNA, or Id-encoding recombinant adenosine virus all fail to elicit 38C13 Id-specific CD8+ CTLs. Thus we do not believe that the CTL-mediated tumor immunity following mAb-opsonized 38C13-DC vaccination is directed at Id-derived epitopes. Rather, it appears that one or more non-Id antigens serve as targets for the induced antilymphoma immunity.

Vaccination with whole tumor cells or antigens shared by normal tissues rather than a defined tumor-specific antigen carries the risk of inducing autoimmunity. Thus, a third important objective of our study was to determine whether mAb-opsonized whole tumor–loaded DC vaccines would induce tumor immunity without triggering systemic autoimmunity. Roskrow et al found that immunization with DCs loaded with lymphoma cell–derived peptides together with CD40 ligand and IL-2–expressing fibroblasts resulted in a severe autoimmune syndrome resembling graft-versus-host disease. In contrast, we found no evidence for autoimmunity in the current study despite the use of whole autologous lymphoma cells. All vaccinated mice rejecting tumor remained healthy when observed for up to 75 days after tumor inoculation, and there was no evidence for autoimmune damage to the splenic B-cell compartment (Figure 7).

We believe that the general strategy of using mAb-opsonized whole tumor–loaded DC vaccines may be more efficacious than simple pulsing of DCs with apoptotic tumor cells or lysates. This approach is amenable to clinical translation with the availability of the antihuman CD20 mAb rituximab. Together, the above results suggest that vaccination with DCs loaded with mAb-coated tumor cells appears to represent a safe, potent, and clinically feasible approach to active cancer immunotherapy that is deserving of assessment in patients with B-cell lymphoma.

The strategy described here could be readily translated into humans with B-cell lymphomas, using rituximab-coated lymphoma cells cocultured with immature monocyte-derived DCs that are induced to mature after antigen loading. To further explore this technique, we are now extending our preclinical studies using murine lymphoma cells engineered by retroviral transduction to express human CD20. Human CD20 may represent an optimal target for tumor cell opsonization given its stable expression on the cell surface without modulation upon antibody binding. Additional studies in this model system using cytokines and other agents to promote optimal maturation and migration of DCs loaded with mAb-opsonized tumor cells may offer techniques for further enhancement of vaccine potency. Furthermore, since the immunity generated by mAb-opsonized tumor-DC vaccination can target non-Id lymphoma antigens, this approach might be combined with Id protein vaccines to obtain additive or synergistic antilymphoma immunity.

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Authorship

Contribution: S.N.F. and K.K.S. designed and performed the research, analyzed the data, and wrote the paper. D.J.B. and K.K. characterized the humoral anti-Id responses; R.E.Y. performed ELISPOT assays; and J.M.T. designed the research, analyzed the data, and wrote the paper.

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