Modeling Dendritic Cell Vaccination for Influenza Prophylaxis: Potential Applications for Niche Populations

Vanaja Konduri,1,a William K. Decker,1,a Matthew M. Halpert,1 Brian Gilbert,2 and Amar Safdar3

1Department of Pathology and Immunology and 2Department of Virology, Baylor College of Medicine, Houston, Texas; and 3Department of Medicine, Division of Infectious Diseases and Immunology, New York University Langone Medical Center

Background. Cancer patients can exhibit negligible responses to prophylactic vaccinations, including influenza vaccination. To help address this issue, we developed in vitro and in vivo models of dendritic cell (DC) immunotherapy for the prevention of influenza virus infection.

Methods. Human cord blood (CB)–derived or mouse splenocyte–derived DCs were loaded with purified recombinant hemagglutinin (rHA). T-cell responses to HA-loaded CB-derived DCs were determined by ELISpot. Protective efficacy was determined by vaccination of BALB/c mice with a single injection of 108 autologous DCs. DC migration to peripheral lymphoid organs was verified by carboxyfluorescein succinimidyl ester staining, and HA-specific antibody titers were determined by enzyme-linked immunosorbent assay. Mice were then challenged intranasally with BALB/c-adapted A/New Caledonia influenza virus derived from four consecutive lung pool passages. Antigen-presenting cell (APC) dysfunction was modeled using the MAFIA transgenic system, in which the Csf1r promoter conditionally drives AP20178-inducible Fas.

Results. CB-derived human DCs were able to generate de novo T-cell responses against rHA, as determined by a system of rigorous controls. Mice vaccinated intraperitoneally developed HA titers detectable at serum dilutions of >1:1000. HA seroconverters survived virus challenge, whereas unvaccinated controls and vaccinated nonseroconverters lost weight and died. Furthermore, use of a model of APC-specific immunosuppression revealed that DC vaccination could generate HA-specific antibody titers under conditions in which protein vaccination could not.

Conclusions. The model demonstrates that DC immunotherapy for the prevention of influenza is feasible, and studies are underway to determine whether populations of immunosuppressed individuals might ultimately benefit from the procedure.

Keywords. dendritic cell; influenza; B-cell malignancy; vaccination.

Influenza is a vaccine-preventable illness associated with serious complications in immunosuppressed patients [1]. Most patients with hematologic malignancy remain susceptible to acute influenza virus infection despite annual vaccination and may develop grave complications after infection [2]. Sequential, conventional vaccine doses have resulted in limited or no improvement in protective responses among patients with hematologic malignancies [3, 4]. Recently, we demonstrated an improvement in protective neutralizing antibody titers in patients with B-cell lymphoma who received a single dose of recombinant hemagglutinin (rHA) that was 9-fold higher than the standard vaccine dose. However, despite improvements in titers among seroconverters, nearly half of the patients enrolled in that study did not respond to the dose [5, 6].

A number of strategies to improve vaccine responses are being evaluated in various populations. Patients with leukemia, patients with lymphoma, and hematopoietic...
stem cell transplant (HSCT) recipients might not develop protective immune responses, in part because of the immunosuppressive effects of the altered cytokine microenvironment, as well as significant deficits in normal dendritic cell (DC) function [7–21]. Such alterations in dendritic cell physiology have fostered the postulate that DC vaccination [22, 23] with hemagglutinin (HA)–loaded DCs could be a means by which to generate effective antigen-specific immunity in patients with immunosuppressive B-cell malignancies. Because little was known about the ability of ex vivo–generated DCs to produce specific immune responses against influenza virus HA, we developed an in vitro model system, using a pure influenza A virus rHA protein in conjunction with umbilical cord blood (UCB)–derived DCs and autologous, predominantly naive [24] UCB lymphocytes. Using this model system, we previously demonstrated by interferon γ (IFN-γ) ELISpot and a 51Cr release assay that functional, HA–specific lymphocytes could be generated. We also mapped an HA–specific, DR15-restricted T–cell epitope and observed tetramer-positive cells [25]. DCs derived from UCB have been shown by us and by others to possess immunostimulatory capacities equivalent to those of adult peripheral blood–derived DCs. The key to the system as an experimental model lies in the nearly complete absence of preprimed memory T cells in neonatal circulation [24–31]. Here, we use this model system to explore T–cell reactivity and cross-reactivity among influenza virus HA derived from 5 different strains. We demonstrate in vivo that vaccination with a single dose of HA–loaded DCs is sufficient to provide protective immunity against lethal influenza A virus challenge and to generate HA–specific antibody titers in a model system that mimics systemic antigen–presenting cell (APC) dysfunction [32].

**METHODS**

**Mice**

Six-week-old BALB/c animals were obtained from Harlan Laboratories (Indianapolis, IN). C57BL/6 mice expressing the MAFIA transgene (Csf1r– driven AP20187–inducible Fas) were obtained from the Jackson Laboratory (Bar Harbor, ME). All mice were maintained in accordance with Institutional Animal Care and Use Committee requirements at Baylor College of Medicine.

**Generation of Immature DCs**

Human DCs were generated from UCB units discarded from the University of Texas MD Anderson Cancer Center Umbilical Cord Blood Bank and approved for research use under an institutional review board–approved protocol. After thawing, cords were diluted 3:1 in CiltinMACS buffer (Miltenyi Biotec, Auburn, CA) supplemented with 0.5% human serum albumin (Baxter, Deerfield, IL), and mononuclear cells (MNCs) were separated by centrifugation on a Histopaque-1077 (Sigma, St. Louis, MO) gradient for 20 minutes at 400 × g. Following purification, mononuclear precursors were separated from lymphocytes by adherence to tissue culture plasticware for 1–2 hours in 50 mL of AIM–V medium (Invitrogen, Carlsbad, CA) supplemented with 10% human AB serum (Atlanta Biologicals, Lawrenceville, GA). Nonadherent lymphocytes were then removed and frozen for later use. Adherent MNCs were cultured for 6 days in 50 mL of AIM–V medium supplemented with 10% human AB Serum, 50 ng/mL granulocyte macrophage colony–stimulating factor (GM–CSF; Amgen, Thousand Oaks, CA), and 10 ng/mL interleukin 4 (IL–4; R&D Systems, Minneapolis, MN). Mouse immature DCs were generated from bone marrow precursors, harvested, and adhered to T300 flasks (Phenix Research, Candler, NC) in AIM–V supplemented with 10% mouse serum (Equitech Bio, Kerrville, TX) and 1% penicillin/streptomycin/amphotericin B (Anti–anti, Invitrogen). The nonadherent fraction was removed after 2 hours, and the adherent fraction was cultured in AIM–V supplemented with 10% mouse serum, anti–anti, 30 ng/mL recombinant murine GM–CSF (R&D Systems, Minneapolis, MN), and 10 ng/mL recombinant murine IL–4 (R&D Systems). For both species, the culture medium was removed and replenished with an equal volume of fresh medium on day 3. Cells were cultured in a humidified chamber at 37°C and 5% atmospheric CO2.

**DC Loading and Maturation**

After 6 days of culture, immature DCs were loaded with one of 5 different HA protein isoforms (Protein Sciences, Meriden, CT) prepared as described previously [5]. Isoforms used included A/New Caledonia/20/99 subtype H1N1 (A/New Caledonia), A/Netherlands/219/03 subtype H7N7, A/Vietnam/1203/2004 subtype H5N1 (A/Vietnam), A/Hong Kong/1073/99 subtype H9N2, and A/Wyoming/3/2003 subtype H3N2. Luciferase enzyme used as a control protein was purchased from Promega (Madison, WI). Immature dendritic cells were suspended at a concentration of 2 × 10^5 cells/mL in Viaspan (Barr Laboratories, Pomona, NY) to improve cell viability during electroporation, mixed with rHA to a final concentration of 50 μg/mL, and incubated for 10 minutes on ice in an electroporation cuvette with a 0.4-cm gap (Biorad, Hercules, CA). Cells were then electroporated at 250 V, 125 μF, and Ω = ∞, using a GenePulser Xcell (Biorad) [26]. Following electroporation, cells were plated in a 6–well plate at 10^6 cells/well in AIM–V supplemented with 50 μg/mL rHA. After 3 hours of incubation in rHA, DCs were matured in AIM–V supplemented with 10% serum, 50 ng/mL (human) or 30 ng/mL (mouse) GM–CSF, 10 ng/mL interleukin 4 (IL–4), 10 ng/mL interleukin 1β (IL–1β; R&D Systems), 10 ng/mL tumor necrosis factor α (R&D Systems), 15 ng/mL interleukin 6 (R&D Systems), and 1 μg/mL PGE2 (Sigma). Mature dendritic cells were phenotyped by flow staining with species–specific anti–CD3, CD11c, CD14, CD80, CD86, CD83, CD209, CD40, and HLA–DR (all from BD Biosciences, Franklin Lakes, NJ). Cells were analyzed on a FACScan flow cytometer (BD Biosciences), using CellQuest software (BD...
Biosciences). All quadrant boundaries used for analysis were based on matched isotype control antibodies.

T-Cell Priming and Restimulation

After 48 hours, loaded human DCs were incubated at a ratio of 1:10 with autologous nonadherent peripheral blood mononuclear cells (typically 50% CD3+) in 1 mL of Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen) supplemented with 10% human AB serum, 1% anti-anti, 2 mM l-glutamine, and 2 ng/mL interleukin 12 (R&D Systems) in a single well of a 12-well tissue culture plate (Corning, Corning, NY). Unused mature DCs were cryopreserved for future use. Starting on day 4, primed lymphocytes were supplemented with 100 U/mL interleukin 2 (IL-2; Chiron, Emeryville, CA), 10 ng/mL interleukin 7 (IL-7; R&D Systems), and 5 ng/mL IL-15 (R&D Systems; IL-T cocktail). Primed lymphocytes were restimulated on day 10 by the addition of thawed DCs. At 24 hours after restimulation, lymphocytes were supplemented with IL-T cocktail. Subsequently, fresh cocktail was added every 48 hours. Following 1 week of expansion in the IL-T cocktail, cells were expanded by the addition of 200 U/mL IL-2 every 48 hours.

Animal Manipulations

Mature BALB/c DCs were harvested and washed twice in phosphate-buffered saline. If DCs were stained with carboxyfluorescein succinimidyl ester (CSFE; Molecular Probes, Eugene, OR), staining was performed for 10 minutes at 37°C according to the manufacturer’s instructions. DCs were resuspended in RPMI 1640 at a concentration of 5–10×10^6 DCs/mL, and mice received a single vaccination of 1–2×10^6 DCs intraperitoneally. Four weeks after vaccination, serum for ELISA analysis was harvested by retroorbital bleed. Six weeks after vaccination, animals were challenged intranasally with 50 μL of a passage 4 BALB/c-adapted A/New Caledonia influenza virus lung pool with a titer of approximately 10^5 median tissue culture infective doses (TCID_{50})/mL. In MAFIA [32] experiments, C57BL/6 animals expressing the MAFIA (Csf1r-EGFP-NGFR/FKBP1A/TNFRSF6) transgene were treated every other day intraperitoneally with 0.006 mg AP20187 (Clontech, Mountain View, CA) or sham injection, beginning 1 week prior to vaccination and ending 4 weeks after vaccination. Depletion of peripheral CD11c^+ cells following AP20187 injection was verified by flow cytometry. After 1 week of AP20187 treatment, mice received a single intraperitoneal injection of either 300 ng rHA protein or 1–2×10^6 rHA-loaded DCs. HA-specific titers were determined 6 weeks after vaccination. Experimental cohorts consisted of 3–5 animals per group, and all experiments were repeated independently on at least 2 separate occasions.

ELISPOT and ELISA

Typically, 10^5–10^6 lymphocyte responders from each culture were mixed at a ratio of 10:1 with thawed DC stimulators and plated in triplicate on anti-IFN-γ–coated ELISPOT plates (BD Biosciences). IFN-γ spots were read with an Axioplan2 Imaging Microscope (Carl Zeiss, Thornwood, NY) and interpreted using KS EliSpot, version 4.5.21, software (Carl Zeiss). Wells containing medium only, DCs only, and unstimulated controls were always blank, and findings have been omitted for simplicity. ELISA was performed on diluted serum samples derived from vaccinated animals as described elsewhere [25].

Hemagglutination Inhibition Assays

These assays were performed as described previously [33, 34].

Sequence Alignments

Amino acid sequence data for each HA isoform were gathered from the GenBank amino acid sequence database (available at: http://www.ncbi.nlm.nih.gov/protein) and aligned with A/New Caledonia by pairwise BLAST analysis (available at: http://blast.ncbi.nlm.nih.gov).

Statistical Analysis

Statistical differences were calculated by an unpaired 2-tailed Student t test unless stated otherwise. Statistical significance of survival following viral challenge was determined by χ^2 analysis with 1 degree of freedom. Statistical significance was defined as a P value of ≤.05. All statistics were calculated using the Microsoft Excel 2003 software package, SP2.

RESULTS

Ex Vivo–Derived DCs Generate De Novo T-Cell Responses Against rHA In Vitro

DCs generated from a single UCB unit were separately loaded with 3 different rHA isoforms or an unrelated control protein (luciferase enzyme) and used to prime and expand naive autologous T cells. After 2 stimulations, expanded T cells were restimulated a third time with differentially loaded DC populations, and IFN-γ secretion was assessed by ELISPOT. As indicated in Figure 1A–D, T-cell populations secreted significant amounts of IFN-γ only when restimulated with the rHA isoform against which they had originally been primed. There was mild, unidirectional cross-reactivity between T cells primed with A/Vietnam rHA and restimulated with A/New Caledonia HA; however, no other cross-reactivities were observed. We also demonstrated the promulgation of antigen-specific immune responses against the control luciferase antigen, a noninfectious protein to which the neonate could not possibly have been exposed in utero. The data unambiguously demonstrate that de novo, influenza virus–specific responses can be generated from ex vivo–derived human DCs.
Matured, rHA-Loaded DCs Administered by Vaccination Upregulate Appropriate Costimulatory Markers and Can Migrate to Peripheral Lymphoid Organs In Vivo

Following in vitro experiments with human UCB-derived DCs, an in vivo model was developed to determine the feasibility and efficacy of rHA-loaded DC vaccination. Adherent stem cells were harvested from the long bones of BALB/c mice, and DC differentiation was induced by culture in GM-CSF and IL-4. Following 6 days of culture, immature DCs were harvested and analyzed by flow cytometry. Figure 2A and 2B demonstrate low or absent levels of CD80 and very little expression of CD83 or CD86. After loading with rHA and maturation with a cocktail of potent inflammatory cytokines, virtually 100% of DCs expressed high levels of CD80, with significant fractions coexpressing CD83 and CD86 (Figure 2C and 2D), suggesting that these mature, bone marrow-derived DC might be capable of priming de novo T-cell responses. To demonstrate migration of rHA-loaded DCs to peripheral lymphoid organs, loaded DCs were stained with carboxyfluorescein succinimidyl ester (CFSE) and administered to BALB/c mice via the intraperitoneal route. Twenty-four hours after injection, the inguinal, mesenteric, renal, and lumbar lymph nodes were dissected from vaccinated mice. Lymph node-derived cells were stained with CD11c-APC and analyzed by flow cytometry. Figure 2E indicates that both CD11c+ and CD11c− CFSE+ cells were present in the peripheral lymphoid organs. In the representative experiment shown here, 19% of CFSE+ cells were CD11c+, whereas only 1% of CFSE− cells were CD11c+. Backgating indicated that virtually all CFSE+ cells originated from the monocyte compartment, as defined by forward-scatter and side-scatter characteristics. No CFSE+ cells were observed among animals vaccinated with unstained DCs (data not shown).

Vaccination With Ex Vivo–Derived DCs Generates High-Titer, HA-specific Antibody Responses

Animals were vaccinated on a single occasion with $1 \times 10^6$ rHA-loaded DCs by either the intraperitoneal or subcutaneous routes. Four weeks after vaccination, mice were bled, and serum HA-specific antibody titers were determined. As shown by the representative experiment in Figure 3A, all mice vaccinated intraperitoneally developed high-titer, HA-specific antibody responses detectable at 100-fold dilutions, whereas mice vaccinated subcutaneously tended to seroconvert more sporadically and with lower HA-specific antibody titers. We also analyzed...
immunological cross-reactivity of the A/New Caledonia response with other recombinant hemagglutinin isoforms. As demonstrated in Figure 3B, antibody cross-reactivity with A/Vietnam was maintained at 10-fold and 100-fold serum dilutions, dissipating only at dilutions of >1000-fold. No cross-reactivity was seen between the A/New Caledonia–specific response and any of the other HA isoforms. Even cross-reactivity with A/Vietnam, the most closely related HA isoform used in the study (Table 1), was somewhat surprising, given that amino acid sequence identity between A/New Caledonia and A/Vietnam was only 63%. Antibody functionality of animals with ELISA-positive sera was assessed by a hemagglutination inhibition assay. As demonstrated by Figure 3C, all ELISA-positive sera were able to inhibit hemagglutination of 0.25% turkey red blood cells in the presence of 100 HA U/mL A/New Caledonia [33, 34] at dilution factors of 6 to 9, corresponding to 32–256-fold (25–28) serum dilutions.

Development of HA-Specific Antibody Titer Protects Against Lethal Challenge From BALB/c-Adapted A/New Caledonia

A/New Caledonia was adapted to BALB/c by 4 serial passages of infected lung pools, producing a titer of approximately 10⁶ TCID₅₀/mL (data not shown). Vaccinated mice with HA titers, subcutaneously vaccinated mice with no HA titers, and unvaccinated mice were all inoculated intranasally with 50 μL of BALB/c-adapted infectious lung pool, a dose of approximately 50 000 TCID₅₀. Between postinoculation days 0 and 4, mice with detectable HA titers lost approximately no more than 15% of their preinoculation weight but regained all weight lost by day 11 and subsequently maintained normal health (Figure 4A). Mice that were unvaccinated or failed to seroconvert continued to lose weight after day 4, and all died from disease between days 5 and 16 (mean time to death, 7.5 days). In this experiment, an HA titer detectable at a 25-fold dilution but undetectable at a 250-fold dilution was sufficient to convey 100% protection from death against an otherwise lethal supraphysiologic influenza virus challenge (P < .005; Figure 4B).

Vaccination With Ex Vivo–Derived DCs Mediates Seroconversion in a Model of APC Dysfunction

To investigate the ability of ex vivo–derived DCs to compensate for innate APC dysfunction as is often observed in immunocompromised cancer patients, we developed a model system of immunotherapy in the MAFIA transgenic mouse. The MAFIA mouse conditionally expresses (in macrophages and DCs) a mutant isoform of Fas that dimerizes and becomes functional in the presence of a small-molecule drug (AP20187), inducing ablation of APCs and significant immune dysfunction. In our model system, mice treated every other day with AP20187 or sham injection were vaccinated intraperitoneally with 300 ng rHA protein or 1–2 × 10⁶ rHA-loaded DCs. As demonstrated in Figure 5, sham-treated mice vaccinated with 300 ng rHA...
protein or rHA-loaded DCs seroconverted efficiently; however, among AP20187-treated mice, only mice vaccinated with rHA-loaded DCs demonstrated measureable seroconversion, at a serum dilution of 1:100. Responses to protein among AP20187-treated mice remained statistically identical to those for preimmune sera.

**DISCUSSION**

Given the high risk for postviral superinfections among patients with cancer and recipients of transplants, influenza vaccination of patients with neoplastic diseases and transplant recipients is considered standard of care treatment [35–39]. In general, seroconversion is poor when vaccination occurs <2 weeks prior to myelosuppressive chemotherapy or <3 months

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**Table 1. Sequence Conservation Among rHA Isoforms**

<table>
<thead>
<tr>
<th>Isoform (Subtype)</th>
<th>Percentage Identity</th>
<th>Percentage Conservation</th>
</tr>
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<tbody>
<tr>
<td>A/New Caledonia (H1N1)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A/Vietnam (H5N1)</td>
<td>63</td>
<td>78</td>
</tr>
<tr>
<td>A/Hong Kong (H9N2)</td>
<td>51</td>
<td>65</td>
</tr>
<tr>
<td>A/Wyoming (H3N2)</td>
<td>42</td>
<td>62</td>
</tr>
<tr>
<td>A/Netherlands (H7N7)</td>
<td>40</td>
<td>59</td>
</tr>
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after myelosuppressive chemotherapy [35, 40–42], when vaccination of HSCT recipients is attempted sooner than 6 months after transplantation [35, 43, 44] or when patients have hematopoietic malignancies, like chronic lymphoid leukemia, B-cell lymphoma, or multiple myeloma, that severely impact adaptive immune function [35, 45, 46]. Recent work by Safdar et al indicated that the rate of enhanced seroconversion among patients with non-Hodgkin lymphoma who received a 9-fold higher dose of a subunit vaccine could be up to 50% higher than that among patients receiving standard dose levels (135 μg vs 15 μg); nevertheless, seroconversion was still relatively poor, possibly a reflection of inherent DC dysfunction and an indication that prophylactic approaches other than traditional vaccination will likely be needed to adequately protect these patient populations [5, 6].

Herein, we demonstrate that ex vivo–derived DCs are capable of generating influenza virus–specific responses in vitro and in vivo and that such responses offer full immunological protection from death following a supraphysiologic lethal challenge dose of 50 000 TCID₅₀. Building on previous work [25], we unambiguously demonstrate that ex vivo–derived human DCs can generate de novo influenza virus–specific responses in vitro, assuaging a concern that could have impacted the development of a DC vaccine intended to impart a de novo response. These results are in good agreement with a growing number of reports detailing the generation of antigen-specific responses from naive UCB T-cell populations by means of DCs [27–31]. Furthermore, we developed an in vivo model that demonstrated that a single injection of HA-loaded DCs was sufficient to generate high-titer, HA-specific antibody responses with the ability to protect seroconverters challenged with a lethal dose of murine-adapted A/New Caledonia. The data suggested that such an approach might be feasible in niche populations of cancer patients with hematologic malignancy who do not respond well to traditional prophylaxis. The immune deficiencies associated with chronic lymphoid leukemia, multiple myeloma, and various B-cell lymphomas are poorly understood, and attempts to model such deficiencies would necessarily be speculative. Although there are several mouse models of hematologic malignancy (eg, the C57BL/KaLwRij mouse as a model of myeloma-associated osteolytic bone lesions) [47], such models tend to focus solely on disease progression and may not replicate malignancy-associated immunodeficiencies.
in patients with cancer. With these caveats in mind, we used the MAFIA mouse model [32] to broadly mimic systemic APC dysfunction. By means of this model, we demonstrated that a single injection of ex vivo-derived rHA-loaded DCs could mediate seroconversion in an APC-deficient environment that was unable to support seroconversion in response to protein vaccination. This model might reasonably approximate immune dysfunction observed in patients with B-cell malignancies.

Of note, antibody cross-reactivity was observed between A/New Caledonia HA and the other most closely related HA isoform tested, A/Vietnam. Although both HA isoforms were only 63% identical at the amino acid level, there apparently exist a substantial number of antigenic regions that maintain enough sequence identity to mediate such cross-reactivity. Antibody cross-reactivity in the mouse might have been predicted, based on weak T-cell cross-reactivity observed in the human experiments. Indeed, human T cells primed against A/Vietnam were able to respond to DCs loaded with A/New Caledonia at a level above that of background (Figure 1B), indicating some conserved T-cell epitopes. Interestingly, this cross-reactivity was only observed in a unidirectional fashion. Human T cells primed against A/New Caledonia did not respond to DCs loaded with A/Vietnam at a level above that of background (Figure 1A). Presumably, HLA considerations played a role in the lack of bidirectional cross-reactivity. There were likely a variety of untested haplotypes for which cross-reactivity would have been not only bidirectional, but also observed between A/New Caledonia and some of the other HA isoforms. Such relationships would be hard to predict in advance, given the vagaries of interactions between major histocompatibility complex and peptides.

The successful use of a UCB model of de novo immune response also raises the interesting possibility of using partially HLA-matched UCB DC to vaccinate patients who cannot be properly mobilized or whose immune dysfunction is so severe as to render impractical any attempts at autologous DC production. In the nontransplant setting, such an approach would appear to be viable, as each individual cord unit could supply a copious amount of DCs for vaccination [48] and graft-versus-host disease would not be an issue. Indeed, allogeneic priming of host lymphocytes would result in the opposite scenario—a host-versus-graft effect. It is not envisioned that cord blood DC vaccines would play a significant role in the UCB transplant setting, as these patients undergo transplantation with curative intent and can receive traditional vaccines after adequate immune reconstitution. Although the literature does not yet supply examples of vaccination with allogeneic UCB DCs, a somewhat analogous and novel approach by our group has successfully generated functional, CLL-specific T cells from partially HLA-matched UCB grafts. It is envisioned that this approach might be used in conjunction with allogeneic UCB HSC/T to treat disease relapse or even in the nontransplant setting as a stand-alone cell therapy [49].

In summary, we present data indicating the feasibility, practicality, and usefulness of DC immunotherapy for prophylaxis of infectious disease. A single vaccination was sufficient to protect from a lethal challenge of species-adapted influenza virus in a wild-type rodent model. Such an approach might be appropriate for niche populations of immunocompromised individuals who respond poorly to traditional prophylactic approaches. The approach might be particularly helpful in ameliorating morbidity and mortality in patients with B-cell malignancies who have specific and debilitating immune deficiencies. Studies to evaluate the applicability of this procedure to the clinical setting are ongoing.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


44. Balan S, Kale VP, Limaye LS. A large number of mature and functional dendritic cells can be efficiently generated from umbilical cord blood-derived mononuclear cells by a simple two-step culture method. Transfusion 2010; 50:2413–23.