Human Dendritic Cells Genetically Engineered to Express High Levels of the Human Epithelial Tumor Antigen Mucin (MUC-1)

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ABSTRACT

We have achieved stable high-level expression of a human tumor antigen, epithelial cell mucin (MUC-1), on human dendritic cells (DCs) by retroviral transduction of CD34+ progenitor cells and their subsequent cytokine-induced differentiation into DCs. The process of retroviral transduction did not alter the growth or differentiation of DCs from CD34+ progenitor cells. Immunofluorescence and electron microscopy studies revealed that the expression of mucin was limited to the body of the DCs and was excluded from the cytoplasmic vesicles of the DCs. Furthermore, the expression of mucin on DCs was similar, if not identical, to the nonpolarized expression of mucin found on carcinoma cells. In functional studies, the MUC-1+ transduced DCs were potent stimulators of allogeneic CD4+ T cells and, in fact, were superior to MUC-1- DCs. Thus, MUC-1+ DCs are expected to be a valuable tool in the immunotherapeutic treatment of patients with tumors that express MUC-1.

INTRODUCTION

Recent efforts in cancer immunotherapy have centered around genetic modification of tumors to increase their immunogenicity. A number of investigators have accomplished this through transfection or transduction of tumor cells with cytokines (1), T-cell co-stimulatory molecules (2, 3), or allogeneic MHC molecules (4). Identification and cloning of tumor antigens recognized by cytolytic T cells (5) has allowed the achievement of tumor-specific T-cell responses in vitro (6). This new strategy of using genetically modified DCs expressing tumor antigens allows the immune response to focus on a specific tumor antigen presented in the context of additional immunostimulatory signals not provided by most tumor cells.

DCs pulsed with either synthetic or naturally processed tumor-derived peptides have been used as immunogens in recent studies in murine models, resulting in the eradication of established tumors (7). In humans, this approach is expected to be limited to a relatively small number of previously identified peptides and to cancer patients who express particular class I or II MHC molecules. In contrast, we have focused our efforts on the expression in DCs of whole tumor antigens. Expression of a tumor antigen as an intact protein eliminates the requirement for prior knowledge of the particular peptide(s) capable of being processed and presented by MHC molecules, as long as the immunogenicity and specificity of the tumor antigen has been established. Loading DCs with whole tumor proteins has the same advantage and has been successfully used in murine (8) and human (9) studies. The further advantage of the transduction approach is the stable long-term expression of the antigen by the DC, which allows its presentation to the immune system for longer periods of time, without the concerns about the turnover of preformed peptide/MHC complexes in vivo after immunization. We have chosen to pursue this strategy by stably introducing the epithelial tumor antigen mucin into human DCs by retroviral infection.

Epithelial cell mucin, encoded by the MUC-1 gene, is expressed by adenocarcinomas of the pancreas, breast, and ovary and by several other tumors of ductal epithelial cell origin (10). Mucin is a large, heavily glycosylated (O-linked) molecule expressed on apical surfaces of ductal epithelial cells. It is aberrantly glycosylated on tumor cells, which results in the exposure of the mucin polypeptide core and the unmasking of otherwise cryptic epitopes that can serve as specific tumor antigens (11). The extracellular domain of this transmembrane mucin protein consists of a large number of tandem repeats of 20 amino acids. Tumor-specific epitopes on the tandem repeat polypeptide core have been shown to be recognized by human T cells (12–14), as well as by antibodies (15, 16). In contrast to other tumor antigens, the recognition of MUC-1 by tumor-specific T cells is that of an intact cell surface protein rather than that of a processed antigen presented by class I or II MHC molecules (MHC-unrestricted recognition) (11). However, these T-cell responses in cancer patients appear weak and ineffective, presumably because they were primed suboptimally with mucin expressed on tumor cells rather than on “professional” APCs such as DCs. We have previously been successful in using genetically modified EBV-immortalized B cells that express MUC-1 on their cell surface as APCs in vitro (17) and in vivo in chimpanzees (18) to stimulate MHC-unrestricted T-cell responses to MUC-1. We hypothesize that using DCs (superior in their T-cell stimulatory capacity to B cells), genetically modified to express MUC-1, could lead to a greatly enhanced immune response to mucin and regression or elimination of the epithelial tumor itself. Furthermore, not only would the transduced DCs express native MUC-1 molecules on their cell surface, thus mimicking tumor cells, but also they would be expected to present potential mucin peptide epitopes on their class I or class II MHC molecules. Mucin expressed on human tumor cells has not yet been shown to generate class I or II restricted responses, possibly because they might require greater levels of T-cell co-stimulation than does the MHC-unrestricted recognition of mucin.

As we had been unsuccessful in stably transducing or transfecting DCs derived from peripheral blood cells, we designed an alternative approach that successfully generates human DCs from CD34+ progenitor cells that express transduced molecules. We demonstrate...
efficient transduction of CD34+ progenitor cells with a retroviral vector encoding the MUC-1 cDNA, followed by the induction of the cells to differentiate into MUC-1 DCs through the use of recombinant human cytokines. This method for reproducible and efficient retrovirally mediated delivery into human DCs of specific tumor-associated antigens can also be used for other important antigens or other molecules, such as cytokines, that can enhance or modify the immune response.

**MATERIALS AND METHODS**

**Isolation and Purification of CD34+ Progenitor Cells.** Umbilical cord blood was obtained from discarded placenta following normal deliveries, with informed consent and Institutional Review Board approval. Cord blood cells were diluted 1:2 with RPMI 1640 (Sigma Chemical Co., St. Louis, MO) and applied to a sodium diatrizoate-Ficoll (Organon Teknika, Durham, NC) gradient. The resulting mononuclear cell fraction was washed twice in PBS (Life Technologies, Inc., Grand Island, NY) containing 1% BSA (Sigma) and resuspended at 1–2 × 10⁶ cells/ml. CD34+ purity was confirmed on a FACScan flow cytometer (Becton Dickinson) as described below using an anti-CD34 mAb (Becton Dickinson, San Jose, CA).

**Growth and Differentiation of DCs from CD34+ Progenitor Cells.** CD34+-enriched cells were plated at 2 × 10⁶ cells/ml in two ml of RPMI 1640 (Sigma) supplemented with 10% FCS, penicillin/streptomycin (100 units/ml penicillin G sodium and 100 mg/ml streptomycin sulfate; Life Technologies, Inc.), and 2 mM L-glutamine (Life Technologies, Inc.); DC medium). The cells were cultured with the recombiant human cytokines GM-CSF (500 units/ml; Schering-Plough, Kenilworth, NJ), TNF-α (100 units/ml; Genzyme, Cambridge, MA), IL-4 (400 units/ml; Immunex, Seattle, WA), and SCF (10 ng/ml; R & D Systems, Minneapolis, MN). These cytokines were added to the cultures every 48 h and the cells expanded as necessary; either the cells had reached confluency or the medium had turned acidic.

**Retrovirus Production.** A cDNA encoding the MUC-1 gene (22 tandem repeats) was cloned into the retroviral vector MFG (MFG-MUC-1). Briefly, MFG is a simplified retroviral vector derived from Moloney murine leukemia virus and does not contain a drug resistance marker. In addition, this vector does not express any other potential antigenic products other than the cDNA of interest. Amphototropic BING producer cells (20) were cultured in DMEM supplemented with 10% FCS, penicillin/streptomycin, 5 mM HEPES, and 2 mM L-glutamine (Life Technologies, Inc.). Approximately 2 × 10⁶ BING cells were transfected with 10 μg MFG-MUC-1 (or MFG-LacZ) plasmid DNA (19, 21) and 2.5 μg of a helper plasmid encoding retroviral gag, pol, and env genes using calcium phosphate precipitation in the presence of 25 mM chloroquine. Tumor virus supernatants were prepared by the transfection of BING cells with only the helper plasmid and were used for mock transductions. Cells were incubated at 37°C for 7–11 h. The medium was replaced and collected after 48 h. The retroviral supernatants were filtered through a 0.45 μm filter and stored at -80°C. Further characterization of MFG-MUC-1 retroviral vector and MUC-1 protein expression on transduced cells is described in more detail elsewhere (R.A. Henderson et al., manuscript in preparation).

**CD34+ Cell Transduction.** CD34+-enriched cells at a concentration of 2 × 10⁶ cells/ml were incubated overnight in DC medium with cytokines. Cells (2–4 × 10⁶) were aliquoted to 15-ml round-bottom culture tubes and resuspended in 1 ml of retroviral supernatant with cytokines and 6–8 μg/ml protamine sulfate. The cells were transduced for 2 h at 26–28°C while centrifuging at 2400 × g. The retroviral supernatant was then removed, and the cells were resuspended in DC medium with cytokines. The transduction process was repeated 24 h later. Nontransduced controls were obtained by using mock-transfected cell supernatants in the place of infectious retroviral supernatants.

**FACS Analysis and FACS Sorting of DCs.** The mAbs specific for HLA-DR, CD4, CD14, CD80 (B7.1), CD18 (LFA-2), CD11a (LFA-1a), CD11b (CR3), CD11c (CR4), CD21 (CR2), CD56 (ICAM-1; Becton Dickinson), CD32 (FcγRII), CD54 (ICAM-1; Becton Dickinson), CD38 (FcyRII), CD45 (FITC conjugated; Ortho Diagnostic Systems, Raritan, NJ), CD1a, CD1b, CD1c, CD58 (AMAC), CD35 (CR1), CD64 (FcγRI), Biosource International, Camarillo, CA), CD86 (B7.2), CD40 (PharMingen, San Diego, CA), CD83, CD23 (FceRII), CD50 (ICAM-3), and CD102 (ICAM-2; Fifth International Leukocyte Differentiation Antigen Workshop) were obtained as purified unconjugated mAbs or as direct conjugates to FITC or PE. W6/32 hybridoma specific for HLA-A,B,C was obtained from American Type Culture Collection. The mAbs BC2, HMFG-2, and SM3, which are specific for mucin were generously supplied by Dr. J. F. McKenzie, Austin Research Institute, Melbourne, Australia and Dr. J. Taylor-Papadimitriou, Imperial Cancer Research Fund, London, United Kingdom. Goat antimon IκBα (β)-PE; Biosource International) was used as a secondary antibody for the unconjugated mAbs listed above. Appropriate isotype control mAbs (Becton Dickinson) were used in all experiments. Briefly, cells were washed once in PBS containing 5% FCS, 2% human AB serum, 1% goat serum, and 0.01% sodium azide (FACS medium). Approximately 1 × 10⁶ cells were plated into individual wells of a 96-well U-bottomed plate and incubated with an appropriate mAbs at 4°C for 30 min. The cells were then washed three times with FACS medium and fixed with 1% formaldehyde before analysis by flow cytometry (FACSscan, Becton Dickinson).

For cell sorting experiments, the cells were stained sequentially for 30 min with 1 ml of the BC2 mAb ascites diluted 1:100 with FACS medium (no sodium azide), 1 ml of a PE-conjugated secondary antibody diluted 1:100, 1 ml of 20 μg/ml mouse IgG, and 1 ml of CD1a-FITC (Ortho) diluted 1:10. The cells were washed extensively and resuspended in 4 ml of 1% FCS/PBS. Stained cells were sorted on a Facstar Plus instrument (Becton Dickinson) into CD1a+ MUC-1+ and CD1a+ MUC-1- populations. The cells were collected during the sort into 20% FCS/RPMI 1640, washed once, and were either used directly or cultured overnight in DC medium.

**Immunofluorescence and Electron Microscopy.** For immunofluorescence microscopy, the cells were stained as above using the antibodies BC2, a Cy3-conjugated goat antimouse IgG(±H+L) secondary (Jackson ImmunoResearch Laboratories, Inc., Westgrove, PA), and CD1a-FITC (Ortho). After staining, the cells were washed and resuspended at 1 × 10⁶ cells/ml in 40% FCS/RPMI 1640 and incubated at 37°C for 5 min. Approximately 1 × 10⁶ cells were applied to standard glass microscope slides at 600 rpm for 6 min using a cytosin cell preparation system (Shandon, Inc., Pittsburgh, PA). The labeled cytosins were mounted, coverslipped, and observed using a Nikon FXA photomicroscope. The slides were visualized using a FITC/Cy3.18 dual pass cube (Omega Optical), a ×60 Planachromat objective, and a ×2 intermediate enlarging lens. Images were collected using an integrating Sony three-chip video camera in conjunction with the Optimas (Bioscan) image analysis package.

For electron microscopy, the cells were stained with control murine IgG, anti-CD1a (AMAC), or antiinum (BC2) mAbs. The cells were washed extensively and then stained with a 5-nm goat antimouse gold immunocconcjugate (Amersham). The cells were gently pelleted and fixed in 2.5% glutaraldehyde in PBS. Following standard electron microscope processing and sectioning, unstained grids were examined using a JEOL 100CXII electron microscope (JEOL, Ltd., Tokyo, Japan). To describe the general morphology of the cells, micrographs were taken at ×2,000, and to examine the localization of antigens, micrographs were taken at ×19,000. The work was performed at the Structural Biology Imaging Center of the University of Pittsburgh Cancer Center, Pittsburgh, PA.

**Allogeneic T-Cell Proliferation Assays.** Serial 1:3 dilutions of the indicated irradiated (3000 rad, ¹³⁷Cs source) stimulator cells were cultured with 2 × 10⁶ allogeneic CD4+ T cells in triplicate for 5 days in 96-well U-bottomed plates (Costar). One μCi of [³H]-thymidine (Amersham, Arlington Heights, IL) was added to each well for the last 1 h of culture. The cultures were harvested using a cell harvester (Skatron, Inc., Sterling, VA) and radioactive incorporation was measured using a β scintillation counter. Allogeneic CD4+ T cells were isolated from human blood obtained from buffy coats purchased from the Central Blood Bank of Pittsburgh (Pittsburgh, PA). Briefly, mononuclear cells were isolated by centrifugation over Ficoll (Organon Teknika), with the monocytes being removed by plastic adherence. The nonadherent cells were stained with tissue culture supernatants of hybridomas specific for CD19 (kindly provided by Dr. Ron Levy, Stanford University, Stanford, CA), CD16 (3G8, provided by the University of Pittsburgh Hybridoma Facility), CD56 (Becton Dickinson), and CD8 (3B5, kindly provided by Dr. Edgar Engleman, Stanford University, CA), washed extensively, and stained with either anti-mouse IgG (Zymed, South San Francisco, CA) coated plastic Petri dishes at 4°C. Nonadherent cells were removed by gentle washing with 5% HS/PBS and
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were routinely >95% CD4+ T cells. Purified allogeneic macrophages were isolated by centrifugal elutriation and were judged to be 85% pure by anti-CD14 staining.

RESULTS

Transduced CD34+ Progenitor Cells Differentiate into MUC-1-expressing DCs. Several different cytokine combinations have been reported to induce differentiation of DCs from precursor cells in bone marrow or peripheral blood (22–24). We compared two cytokine combinations, GM-CSF/TNF-α and GM-CSF/IL-4, for their ability to generate DCs from CD34+ progenitor cells and for the role that SCF played in that process. The combination of GM-CSF and TNF-α was very efficient in generating CD1a+ DCs and, surprisingly, the combination of GM-CSF and IL-4 also produced appreciable numbers of DCs (Fig. 1, Table 1). Unlike GM-CSF/TNF-α, the generation of DCs with GM-CSF/IL-4 appeared to require the presence of SCF (Fig. 1). In addition, the DCs grown with IL-4 expressed higher levels of CD1a and did not express CD14 (data not shown). On the basis of these results, the cytokine combination for the growth of DCs was GM-CSF and TNF-α and IL-4, with the addition of SCF only in the first week of culture. This combination allowed the maximal proliferation of DCs while retaining the CD1a+CD14– phenotype induced by IL-4 (Fig. 2 and Table 2).

Table 1 CD1a+ DCs isolated from CD34+ progenitor cells using varying recombinant human cytokines

<table>
<thead>
<tr>
<th>Cytokine Combination</th>
<th>GM-CSF and TNF-α</th>
<th>GM-CSF and IL-4</th>
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<tbody>
<tr>
<td>No SCF</td>
<td>71%</td>
<td>63%</td>
</tr>
<tr>
<td>SCF (5 days)</td>
<td>52%</td>
<td>67%</td>
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Expression is measured as mean fluorescence intensity of cells labeled with the antihuman CD1a antibody conjugated to FITC and analyzed by flow cytometry.

Table 2 Cell surface phenotype of transduced or mock-transduced human DCs at day 14 of culture

<table>
<thead>
<tr>
<th>Cytokine Combination</th>
<th>Mock (56.7%)</th>
<th>MFG-LacZ (56.4%)</th>
<th>MFG-MUC-1 (57.3%)</th>
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</thead>
<tbody>
<tr>
<td>CD1a</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>CD1b</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
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<tr>
<td>CD1c</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>CD4</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>MHC molecules</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>HLA-A, B, C</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Co-stimulatory molecules</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>CD80 (B7.1)</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>CD86 (B7.2)</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
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<tr>
<td>CD40</td>
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<td>Lineage</td>
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<td>CD1a-FITC</td>
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<td>CD54 (ICAM-1)</td>
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</tr>
<tr>
<td>CD50 (ICAM-3)</td>
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</tr>
<tr>
<td>CD58 (LFA-3)</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>CD11a (LFA-1a)</td>
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<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>CD18 (LFA-1β)</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
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<tr>
<td>Complement receptors</td>
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<td>+ + +</td>
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<tr>
<td>CD35 (CR1)</td>
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<td>+ + +</td>
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<tr>
<td>CD21 (CR2)</td>
<td>+ + +</td>
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<td>CD11b (CR3)</td>
<td>+ + +</td>
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<tr>
<td>CD11c (CR4)</td>
<td>+ + +</td>
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</table>

Expression is measured as mean fluorescence intensity of cells labeled with the antihuman mAbs listed and analyzed by flow cytometry. DCs were analyzed using two-color FACS analysis using the antibodies CD1a-FITC, and those listed in the table which were conjugated to PE. The DCs were gated based on both size (forward angle light scatter) and CD1a expression.

*+*, a 1-log increase in mean fluorescence intensity above background due to staining with control antibody; ---, mean fluorescence intensity equivalent to control antibody; --/+ , heterogeneous expression.
To generate DCs that express MUC-1, CD34+ cells were purified from umbilical cord blood and transduced as described in "Materials and Methods." Cultures of CD34+ cells transduced with either MFG-MUC-1 or MFG-LacZ retroviral vectors grew as well as the control cultures that were mock transduced (Fig. 2). In addition, both cultures produced similar numbers of DCs as judged by the percentage of CD1a+ cells in each culture (Table 2). Inasmuch as the superior APC function of DC depends on the expression of specific cell surface proteins, we compared their level of expression on transduced and nontransduced cells. There was no difference between the transduced DCs (MUC-1 or LacZ) and mock-transduced controls in any of the cell surface proteins analyzed (Table 2). These results indicate that retroviral transduction of the progenitor cells did not alter their growth and differentiation or the cell surface phenotype of the resulting DCs.

The transduced DCs were analyzed for their expression of mucin by two-color FACS analysis using FITC-labeled antibody against CD1a and a panel of mAbs against various differentially glycosylated forms of MUC-1 (PE-labeled secondary antibody; Fig. 3A). This analysis
showed that approximately 15% of the DCs expressed mucin at high levels as determined by staining with the antimucin antibody BC2 (Fig. 3A). This antibody recognizes all forms of mucin regardless of the state of its glycosylation. There was only weak staining with the antimucin antibodies SM3 and HMFG-2 (Fig. 3A). These two antibodies preferentially recognize epitopes on mucin that are formed by the aberrant glycosylation of the mucin protein as it is expressed on tumor cells. These epitopes are closely associated with the recognition of mucin by CD8 \(^+\) mucin-specific T cells (11).

A separate culture of the transduced DCs was incubated for 48 h before staining in the presence of 10 mM GalNac, an inhibitor of O-linked glycosylation (Fig. 3B). This treatment was previously shown to induce expression of tumor-associated mucin epitopes recognized by antibodies SM3 and HMFG-2 (25). GalNac-treated DCs continued to exhibit strong reactivity with the antibody BC2, but now they also stained well with the tumor-specific antibodies SM3 and HMFG-2 (Fig. 3B). These results indicate that the mucin expressed by transduced DCs can be modified to express tumor-specific epitopes, thus mimicking the mucin protein as it is expressed on tumor cells and recognized by patients' antibodies and T cells. DC viability, yield, and cell surface phenotype were unaffected by the treatment with GalNac. There was no significant staining with any of the antimucin antibodies of DCs transduced with MFG-LacZ or mock transduced, regardless of whether they were treated with GalNac or not. The transduction of DCs with the control vector MFG-LacZ was confirmed by X-Gal staining.

The percentage of CD1a \(^+\) DCs that expressed high levels of the mucin protein by FACS analysis over multiple experiments was determined to be 13.5 ± 1.9%. This percentage was similar to that observed in CD34 \(^+\) cells immediately following the transduction procedure (data not shown), indicating that the expression of the mucin protein was stable throughout the culture period and the subsequent differentiation of DCs. This value may be an underestimate in that only cells expressing high levels of mucin were included in the calculation. In previous experiments, we have determined that only 50% of 3T3 cells transduced with MFG-MUC-1 express mucin at high levels, and thus the overall percentage of DCs expressing mucin may be closer to 27%.

**MUC-1 Cell Surface Distribution and Expression on DCs Is Similar to That on Carcinoma Cells.** Normally, the mucin protein is found only on the apical cell surface of glandular epithelial cells. However, in carcinoma cells, the polarization of the protein is lost and expression is often increased compared to normal cells (10). To further characterize expression of mucin by DCs, we analyzed the distribution of the protein on the cell surface using both immunofluorescence (Fig. 4) and electron microscopy (Fig. 5).

By immunofluorescence, mucin shows uniform punctate stain over the DC surface (Fig. 4). Interestingly, mucin expression appears to be restricted to the body of the DCs because the cytoplasmic veils of the DCs do not show labeling. On the other hand, CD1a is uniformly located over the entire cell surface, including the cytoplasmic veils (Fig. 4).

At the ultrastructural level, a similar pattern was observed (Fig. 5). The mucin protein is found in discrete "patches" restricted to the cell surface (Fig. 5D), whereas CD1a is evenly distributed on both the cell surface and the cytoplasmic veils (Fig. 5C). The microscopic characterization of mucin cell surface expression and distribution shows that expression of mucin on transduced DCs is very similar to the nonpolarized expression of mucin on carcinoma cells (26).

**MUC-1 + DCs Are More Potent Allogeneic T-Cell Stimulators than MUC-1− DCs.** The extracellular domain of mucin is a rigid rod-like structure that projects high above the plasma membrane (200–500 nm) (27). Although previous work in our laboratory has demonstrated that mucin can be a target for recognition by CD8 \(^+\) mucin-specific T cells, a substantial body of evidence exists that the mucin protein can also exert antiadhesive effects and can inhibit recognition of carcinomas by alloreactive T cells (28, 29). The possibility existed that the expression of mucin on transduced DCs might inhibit stimulation of T cells by the DC by either preventing T cell-APC conjugate formation or by blocking specific molecules, such as MHC or co-stimulatory molecules, from interacting with their ligands.

To test whether the expression of mucin could interfere with the stimulatory capacity of DCs, transduced cells were sorted by flow cytometry into two DC populations, CD1a\(^+\) MUC-1\(^−\) and CD1a\(^+\) MUC-1\(^+\). Both populations were compared to macrophages for their ability to stimulate allogeneic T cells in a 5-day [\(H^3\)]-thymidine incorporation assay. In two independent experiments, both populations of DCs were substantially better than macrophages in their ability to stimulate T-cell proliferation (Fig. 6). Unexpectedly, the MUC-1\(^+\) DCs were better at stimulating allogeneic T cells than were the MUC-1\(^−\) DCs (Fig. 6).

**DISCUSSION**

A large number of investigators have tried to improve the immune response to tumors by providing them with additional immunostimulatory signals such as cytokines or co-stimulatory molecules. Although these approaches have been successful in some murine tumor
models, it is not clear whether they will be equally successful in cancer patients. In addition, there exists the potential for eliciting autoimmunity against normal tissue specific antigens together with immunity against presumptive tumor antigens. With the identification and cloning of tumor antigens recognized by T cells, it is now possible to transfer tumor antigens into professional APCs. This would allow the tumor antigen to be presented exclusively to the immune system with multiple, presumably optimal, immunostimulatory signals. Stable transduction or transfection of DCs grown in vitro has not yet been reported. We have demonstrated in this report the feasibility of an alternative and more successful approach to stably introduce genes into human DCs. We have transduced human CD34+ progenitor cells with the retroviral vector MFG-MUC-1 (encoding the epithelial tumor antigen mucin) and differentiated them into DCs. The transduced DCs express high levels of MUC-1 with a cell surface distribution similar to that on carcinomas. Furthermore, the expression of tumor-specific mucin epitopes can be induced on the transduced DCs by treating the cells with an inhibitor of O-linked glycosylation. Importantly, the process of retroviral transduction and expression of the transduced genes does not alter or influence the generation or differentiation of DCs from CD34+ progenitor cells. Although the levels of transduction are not as high as might be desired, the tremendous expansion (~100-fold) of DCs from only a small number of CD34+ cells will allow the generation of large numbers of transduced DCs. A single case of transient transfection of a tumor antigen into DCs was recently reported (30). In contrast to that report, we show here that retrovirally mediated transduction results in stable and long-term expression of a tumor antigen. This may be an important difference because future immunotherapy using DCs may be more effective if the DCs are capable of prolonged rather than transient expression and presentation of antigen to tumor-specific T cells in vivo.

An unexpected finding was that the MUC-1+ DCs stimulated allogeneic CD4+ T cells to a greater degree than MUC-1− DCs. It may be that the cross-linking of MUC-1 with antimucin antibodies during the cell sorting process served to activate the DCs, as has been described previously for the activation of DCs by crosslinking of
CD40 (31). Indeed, mucin was recently shown to have signaling properties when crosslinked on carcinoma cells (32). Direct examination of this possibility has been precluded in these experiments by the low number of MUC-1+ DCs available after cell sorting. An alternative explanation is that MUC-1 interacts with some as yet unidentified T-cell surface molecule to cause enhanced T-cell proliferation. This phenomenon, however, has never been observed previously in T cell-tumor cell interactions. These issues will be addressed in future experiments using antitimucin antibodies or soluble mucin protein to block potential interactions. Regardless of the precise mechanism, this fortuitous finding is expected to facilitate the priming and activation of MUC-1-specific T cells.

We have demonstrated previously that tumor-associated mucin can be recognized directly by tumor-specific MHC-unrestricted CD8+ T cells (11). This recognition is not of a processed peptide, but rather of the intact cell surface protein, and it appears to be dependent upon the aberrant glycosylation of the mucin protein on tumor cells, the tandem repeat nature of the molecule, and the loss of polarized expression. Consequently, we have focused our efforts on expressing the mucin protein in DCs as a cell surface protein to best mimic the nature of the antigen as it is found on tumor cells. At the outset of this study, it was not clear whether we would be able to stably express the mucin protein in DCs as a cell surface protein to best mimic the nature of the antigen as it is found on tumor cells. The ability of DCs to stimulate strong primary allogeneic T-cell responses would not allow the discrimination between allogeneic and MUC-1-specific T-cell responses.

Retrovirally mediated transduction of CD34+ cells as a means of expressing proteins in DCs is not limited to tumor antigens. Genes for antigens derived from either viral or bacterial pathogens can also be transduced, as can other molecules that are important for influencing the DC function. For example, transducing either IL-12 or IL-4 cytokines into DC can direct the immune response to a predominately Th1 or Th2 type, respectively. The ability to generate transduced DCs with stable expression of tumor antigens, other foreign antigens, or selected cytokines represents an important step in the development of new immunotherapeutic approaches to the treatment of cancer and other diseases.

ACKNOWLEDGMENTS

The authors thank Amy Kemp, Jason Lancia, Lori McKensie, and William Konitsky for expert technical assistance. The authors also thank Dr. Warren Pear for the BING amphoteric packaging cell line, Dr. Mitchell Finer for the plasmid encoding retroviral genes, and Dr. Albert Donnenberg and Vera Donnenberg for their invaluable help and assistance on FACS analysis.

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