Cutting Edge: Physical Interaction Between Dendritic Cells and Tumor Cells Results in an Immunogen That Induces Protective and Therapeutic Tumor Rejection

Christina M. Celluzzi and Louis D. Falo, Jr.

*J Immunol* 1998; 160:3081-3085; ;

http://www.jimmunol.org/content/160/7/3081

**References**

This article cites 28 articles, 11 of which you can access for free at:
http://www.jimmunol.org/content/160/7/3081.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Cutting Edge: Physical Interaction Between Dendritic Cells and Tumor Cells Results in an Immunogen That Induces Protective and Therapeutic Tumor Rejection

Christina M. Celluzzi* and Louis D. Falo, Jr.2*†

Dendritic cells (DCs) are potent professional APCs capable of presenting Ag in the context of costimulatory signals necessary for T cell activation. Although tumor cells express target Ags, they are generally incapable of stimulating an immune response. We show that the short term physical interaction of DCs and tumor cells, with or without cell fusion, results in rapid, efficient, and stable DC-tumor cell association. Immunization of naive mice with unselected, irradiated DC-tumor cell conjugates induces tumor-specific CD8+ cytotoxic T cells and protection from lethal tumor challenge. Furthermore, the immunogenicity of this cellular vaccine is dependent on the physical interaction of DCs and tumor cells before injection. Immunization with DCs and tumor cells after physical interaction can result in the regression of established tumors and persistent antitumor immunity. These results suggest that immunization with DC-tumor cell vaccines may be a simple, rapid, and potent strategy for tumor immunotherapy. The Journal of Immunology, 1998, 160: 3081–3085.

Cutting Edge: Physical Interaction Between Dendritic Cells and Tumor Cells Results in an Immunogen That Induces Protective and Therapeutic Tumor Rejection

Cytoxic T lymphocytes are a critical component of the immune response to tumors. CTL responses are sufficient to protect against tumors and can eliminate even established cancers in murine tumor models and in humans (1). Inducing strong Ag-specific CTL responses is the goal of many current cancer vaccine strategies. The development of CTL-dependent antitumor immunization strategies depends on both the identification of tumor Ags recognized by CTLs and the development of methods for effective Ag delivery.

Part 1

CTls target tumors through recognition of a ligand consisting of a self MHC class I molecule and peptide Ag generally derived from proteins synthesized within the tumor cell (2, 3). However, for CTL induction and expansion to occur, the antigenic ligand must be presented to CTLs in the appropriate context of costimulation usually provided by professional APCs (4). Delivery of exogenous Ag to the endogenous MHC class I restricted processing pathway of professional APCs is a critical challenge in cancer vaccine design. Ag delivery strategies currently under development include immunization with defined peptides (5), particulate proteins capable of accessing the class I pathway of professional APCs in vivo (6), heat shock proteins isolated from tumor cells (7), or adoptive transfer of Ag-loaded APCs (8–12). In addition, recent studies suggest that DNA vaccines encoding tumor Ags delivered by viral vectors or liposomes, or as naked DNA, can induce potent antitumor immunity (13–15).

In addition to the challenge of Ag delivery, most current tumor immunization strategies depend on the identification and production of appropriate tumor Ags. To overcome this limitation, tumor cells themselves may be used as immunogens. It is likely that a tumor cell expresses a set of tumor-specific peptide-MHC complexes recognized by T cells. However, progressive tumors are generally nonimmunogenic at least in part because they are incapable of providing costimulation. Engineering tumor cells to provide APC function could potentially result in polyvalent immunization to multiple tumor-specific epitopes, while obviating the need to identify specific tumor Ags. A variety of strategies are being developed to provide “APC-like” function to tumor cells primarily by transfecting tumor cells with genes encoding costimulatory molecules or cytokines (reviewed in Ref. 16). However, recent studies demonstrate that the in vivo generation of immune responses against tumor cells generally occurs through cross-priming, with tumor Ag presentation being dependent on bone marrow-derived APCs of the host (17). The mechanism by which tumor Ags are taken up and presented by host APCs remains unclear.

Dendritic cells (DCs) are the most potent APCs identified thus far, and adoptive transfer of Ag-loaded DCs can induce effective CTL-dependent antitumor immunity (8–12). Recent advances have made it possible to obtain significant quantities of dendritic cells from bone marrow or peripheral blood-derived precursors (18, 19). The efficacy of DC adoptive transfer therapies suggests that in vitro manipulated DCs maintain essential APC functions.

*Department of Dermatology and †University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Received for publication December 12, 1997. Accepted for publication January 23, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grants IR29CA70318 from the National Institutes of Health, by Research Project Grant 97-160-01 from the American Cancer Society (L.D.F.), and by a grant from Cheesbrough Ponds USA through the Dermatology Foundation (C.M.C.).

2 Address correspondence and reprint requests to Dr. Louis D. Falo, Jr., Department of Dermatology, University of Pittsburgh School of Medicine, 190 Lothrop Street, Pittsburgh, PA 15213. E-mail address: LoD2@pitt.edu

Copyright © 1998 by The American Association of Immunologists 0022-1767/98/$02.00

Abbreviations used in this paper: DCs, dendritic cells; PEG, polyethylene glycol.
including appropriate trafficking and localization, and Ag presentation in the context of requisite costimulatory signals for T cell induction. DC adoptive transfer therapies are currently limited by their dependence on in vitro Ag loading and the availability of appropriate, defined tumor Ags.

In a novel and promising approach to tumor cell-based immunization, Guo et al. (20) have shown that the fusion of activated B cells to tumor cells produces a potent immunogen, capable of inducing tumor-specific tumor immunity. Like B cell-tumor cell fusion products, the product of DC-tumor cell fusions is also a potent immunogen (21). The broad applicability of APC-tumor cell fusion strategies to human tumor immunotherapy will likely depend on both the capacity to generate and select immunogenic APC-tumor cell hybrids and the stability of their expression of the factors critical to immunogenicity. Here we show that short term coculture of DCs and tumor cells, with or without prior fusion, results in a potent immunogen capable of inducing CTL-mediated protective antitumor immunity and the regression of established tumors. Our results suggest that the immunogenicity of this cellular vaccine is dependent on the physical interaction of DCs and tumor cells before injection. The implications of these findings for human tumor immunotherapy and vaccine design are discussed.

Materials and Methods

Mice and cell lines

Female C57BL/6 mice, 5 to 8 wk old, were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed at the Central Animal Facility of the University of Pittsburgh. B16 is a C57BL/6-derived melanoma (H-2b) (American Type Culture Collection (ATCC), Rockville, MD). 3LL is a C57BL/6-derived lung carcinoma (22). Cell lines were maintained in Dulbecco’s modified Eagle’s medium containing 10% FCS and antibiotics.

Antibodies

mAbs used to deplete cell subsets were prepared from the hybridomas GK1.5 (anti-CD4, ATCC TIB 207), 2.43 (anti-CD8, ATCC TIB 210), 30-H12 (anti-Thy-1.2, ATCC TIB 107), B220 (anti-B cell surface glycoprotein, ATCC TIB 146).

Preparation of DCs

DCs were prepared from bone marrow as described (9), with slight modifications. Briefly, bone marrow cells were depleted of lymphocytes and cultured at 5 × 10^7 cells/ml in 10% FCS-containing RPMI 1640 (Irvine Scientific, Santa Ana, CA) with granulocyte-macrophage-CSF (10^3 U/ml; Sigma Chemical Co., St. Louis, MO). Loosely adherent cells were collected on day 6 for fusion or coculture. DCs obtained by these methods expressed both CD86 (B7.2) and class II MHC Ags as determined by flow cytometry (9, 11) (data not shown).

Fusion or coculture of DCs and tumor cells

Day 6 DCs were fused with B16 or 3LL cells at a ratio of 6:1 using polyethylene glycol (PEG) warmed to 37°C as described (23). After PBS washes, fused cells were cultured overnight at 37°C in RPMI 1640 (10% FCS) containing granulocyte-macrophage-CSF. Cocultured groups were prepared from the hybridomas H12 (anti-Thy-1.2, ATCC TIB 107), B220 (anti-B cell surface glycoprotein, ATCC TIB 146).

Cytotoxicity assay

Splenocytes (30 × 10^6), harvested from mice 7 days after the immunization (see below), were restimulated by coculture with irradiated B16 or 3LL cells (7.5 × 10^6, 20,000 rad) for 5 days. After this time, cytotoxicity assays were performed as described (9). Briefly, target cells were labeled by incubation in RPMI with ^51Cr (100 μCi; NEN, Boston, MA) for 18 h at 37°C, washed, and then cocultured at 2 × 10^5 target cells/well for 4 h at 37°C in 96-well round-bottom plates (200 μl/well) with effector cells at the ratios given in Table I. In some cases, effector cells were depleted of CD4+, CD8+, or Thy-1.2 by incubation with mAbs against these markers plus complement. Collected and counted were 100 μl of supernatants from triplicate cocultures. The SE of the mean of triplicate cultures was not >5%. Data points are expressed as the mean percent specific release of ^51Cr from target cells and were calculated as described (9).

Protection assay

On day 0, C57BL/6 mice were immunized s.c. in both lower flanks (100 μl/mouse) with DCs alone (1.7 × 10^7 per mouse), tumor cells (B16 or 3LL, 3 × 10^7 per mouse) alone, fused DCs and tumor cells (B16 or 3LL, 6:1 (i.e., 1.7 × 10^6 DCs: 3 × 10^6 tumor cells per mouse)), mock-fused (i.e., cocultured) DCs and tumor cells (B16 or 3LL, 6:1), identical numbers of DCs and tumor cells (B16 or 3LL, 6:1) injected together without prior coculture, identical numbers of DCs and tumor cells cocultured (B16 or 3LL, 6:1) in Transwell plates (Costar, Cambridge, MA) to prohibit direct cell contact and then injected, supernatants from the same Transwell cocultures (not shown), or PBS. Cells were irradiated (20,000 rad) and re-suspended in PBS before injection. Seven days later, mice were challenged with tumor cells (B16 or 3LL, 5 × 10^5/mouse/200 μl at 100 μl/mouse) in PBS delivered by intradermal injection to the midflanks bilaterally. Surviving mice that became moribund were killed according to animal care guidelines of the University of Pittsburgh Medical Center. Survival is recorded as the percentage of surviving animals.

Immunotherapy

Mice were challenged intradermally in the midflanks bilaterally with 3LL cells at 5 × 10^5 cells/mouse. On day 7 (average tumor size, 5.9 mm^2/mouse, SE ± 0.8), mice were immunized by s.c. injection bilaterally in the lower flanks with individual or combinations of DCs and tumor cells (listed above). A second injection was given on day 10. Survival was followed as described above.

Results and Discussion

The direct interaction of APCs with tumor cells could result in an effective tumor-specific immunogen, either by conferring sufficient APC function to tumor cells for T cell activation or by facilitating the delivery of tumor Ags to DCs for processing and presentation. To test this hypothesis, we fused DCs with single-cell suspensions of either B16 melanoma cells or 3LL Lewis lung carcinoma cells using PEG by described techniques (23). Alternatively, mock fusions were performed by identical manipulations in the absence of PEG. In both cases, cells were combined at a DC-tumor cell ratio of 6:1 and cocultured for 24 h after manipulation. To determine the efficiency of cell association, DCs and tumor cells were prelabeled with DiIC18(5) or DiOC16(3), respectively, and analyzed by flow cytometry. Analysis of separate, control cell populations after 24 h in culture demonstrated appropriate single-color labeling (DCs, upper left, Fig. 1A; tumor cells, lower left, Fig. 1B) compared with unstained controls (not shown). In contrast, after 24 h of culture, cells from both cocultured and fused groups showed a high degree of association, with most cell conjugates expressing both DiIC18(5) and DiOC16(3) (Fig. 1, C and D). Importantly, most tumor cells were associated with DCs (Fig. 1, C and D). Morphologically, both fused and cocultured groups demonstrated distinct cell aggregates that could not be easily disrupted (not shown). The capacity of DCs to cluster with T cells and other cell types has been previously described (24).

In initial experiments, we sought to determine the capacity of DC-tumor cell conjugates to induce tumor-specific CTLs. Syngeneic naive mice received a single s.c. injection of irradiated DC-tumor cell fusion products without adjuvant. Seven days after immunization, spleen cells were restimulated in vitro and then assayed for lytic activity against tumor targets (9). As controls, groups of mice were immunized with PBS alone, equivalent numbers of irradiated DCs, or tumor cells alone. In addition, some
groups of mice were immunized with identical numbers of irradiated cocultured cells from mock fusions. Splenocytes from mice immunized with tumor-DC fusions demonstrated tumor-specific lytic activity. Effector cells from mice immunized with B16-DC fusions lysed B16 targets, but not the irrelevant tumor EL4 (Table I). Similarly, splenocytes from mice immunized with 3LL-DC fusions demonstrated specific lytic activity against 3LL (Table I). Importantly, effector splenocytes from mice immunized with cells from tumor-DC cocultures that had not undergone fusion demonstrated similar CTL-mediated tumor-specific lytic activity (Table I). Depletion of CD81 or Thy-11 but not CD41 cell subsets from effector populations using mAbs eliminated lytic activity and demonstrated that lysis depended on Thy-11CD81 T cell subsets characteristic of MHC class I restricted CTL effector cells (Table I). The effect of this immunization strategy on NK cell activity in general was not directly addressed. In contrast to immunizations with DC-tumor cell fusions or cocultures, identical numbers of DCs and tumor cells that had not been cocultured but were injected together at a 6:1 ratio did not induce tumor-specific lytic activity (Table I, coinjection groups), suggesting that in vitro coincubation of DCs with tumor cells was necessary for immunogenicity. Furthermore, injection of DCs alone that had been cocultured with tumor cells at identical ratios and cell densities, but separated by a porous membrane barrier to prevent direct DC-tumor cell contact, did not induce lytic activity (Table I), suggesting that tumor-specific immunity was not mediated by the transfer of soluble factors from tumor cells to DCs in these in vitro cocultures.

To determine the capacity of DC-tumor conjugates to induce antitumor immunity in vivo, groups of naive mice were immunized s.c. with irradiated DC-tumor cell conjugates without adjuvant and then challenged 7 days later by intradermal injection of the tumor cells in the flanks bilaterally. Mice immunized with irradiated cells from DC-B16 fusions or cocultures were completely protected from lethal challenge with B16 tumor cells (Fig. 2A). Groups of mice injected with PBS, similar numbers of irradiated DCs or tumor cells alone, or irradiated DCs from membrane-separated Transwell DC-tumor cell cultures were not protected and uniformly developed lethal tumors (Fig. 2A). Importantly, mice immunized with DCs and tumor cells that were injected together

---

**FIGURE 1.** Efficiency of DC-tumor cell association. DiIC-labeled DCs (FL4) and DiO-labeled B16 tumor cells (FL1) were analyzed by flow cytometry to determine the extent of cell association between the two cell populations after fusion or coculture. A, DiIC-labeled DCs alone; B, DiO-labeled B16 cells alone; C, DiIC-labeled DCs and DiO-labeled B16 after coculture; D, DiIC-labeled DCs and DiO-labeled B16 cells after fusion.

---

**Table I.** Immunization with DC-tumor cell vaccines induces tumor-specific CD8+ CTLs

<table>
<thead>
<tr>
<th>Immunization</th>
<th>E:T (25:1)</th>
<th>E:T (50:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16 targets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DCs alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B16 alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B16-DC coinjection</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B16-DC Transwell cultures</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B16-DC fusion</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>B16-DC coculture</td>
<td>65</td>
<td>96</td>
</tr>
<tr>
<td>Thy-1.2 depleted</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>CD8+ depleted</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>CD4+ depleted</td>
<td>56</td>
<td>ND</td>
</tr>
</tbody>
</table>

| 3LL targets                         |            |            |
| PBS                                 | 0          | 0          |
| DCs alone                           | 0          | 0          |
| 3LL alone                           | 0          | 0          |
| 3LL-DC coinjection                 | 0          | 0          |
| 3LL-DC Transwell cultures          | 0          | 0          |
| 3LL-DC fusion                      | 4          | 23         |
| 3LL-DC coculture                   | 10         | 37         |

*Groups of mice were immunized with PBS, identical numbers of DCs alone, tumor cells alone, products of DC-tumor cell fusion, or mock fusions (i.e., cocultures). Some groups of mice were immunized with cells obtained from unfused cocultures of DCs and tumor cells that had been cultured in separate chambers of Transwell plates (Transwell cultures) or with identical combinations of DCs and tumor cells injected together without prior coculture (coinjection). All cellular vaccines were irradiated before s.c. injection. Splenocytes were harvested from mice 7 days later and restimulated in vitro with the tumor cell line used to immunize (B16 or 3LL). Lytic activity of effector cells was determined by assay of 51Cr release from labeled target cells coincubated with effector cells at E:T ratios, of 25:1 and 50:1. To determine the phenotype of effector cells, splenocytes from mice were depleted of CD4+, CD8+, or Thy-1.2+ cells (where indicated) by incubation with mAbs against these markers plus complement and similarly evaluated at E:T ratios of 25:1 for lytic activity. In all cases, <5% lysis was observed against control EL4 tumor targets not used in the immunizations (not shown).
without prior coculture were not protected (Fig. 2B). This is in agreement with previously published results (20, 21). Similarly, immunization with irradiated cells from DC-3LL cocultures protected mice from challenge with 3LL, while s.c. injection of irradiated DCs or tumor cells alone was ineffective (Fig. 2C).

Taken together, these results demonstrate that immunization with DC-tumor cell conjugate vaccines can induce tumor-specific CTLs and potent protective antitumor immunity against two distinct, poorly immunogenic tumors. To determine the therapeutic potential of this immunization strategy, we attempted to induce tumor rejection in mice with established tumors. 3LL tumor cells were injected intradermally into the flanks of naive mice bilaterally (day 0). Seven days later, groups of tumor-bearing mice were immunized with either PBS or irradiated DCs or 3LL cells alone, tumors were progressive with no mice surviving beyond day 33 (Fig. 3A). All mice immunized with irradiated DC-3LL fusion products survived for at least 72 days and had no evidence of tumor when the experiment was terminated (Fig. 3A). Immunization with cocultured cells from mock fusions prolonged survival in most mice and resulted in complete regression in 75% of the animals treated. Furthermore, mice that demonstrated complete tumor regression were rechallenged 3 mo later by i.v. injection of tumor cells. Tumors did not develop in mice that received fusion products or coculture products (Fig. 3B), demonstrating that the antitumor immunity induced by these vaccines was long lasting.

Our studies begin to address the mechanism by which DC-tumor cell conjugate vaccines induce antitumor immunity, but the precise mechanism remains unclear. It is possible that the association of tumor cells with DCs confers sufficient costimulatory function to stimulate T cells specific for Ag-MHC complexes expressed by the tumor cell either by coexpression of costimulatory function and antigenic ligand by a newly formed "hybrid" cell or by complementary contributions of ligand presentation and costimulation by distinct cells in close physical association. It is also possible that intimate association of DCs with tumor cells facilitates the transfer of tumor associated Ags to appropriate Ag-processing pathways of APCs. In this regard, although exogenous proteins generally do not stimulate CTLs because they do not enter the MHC class I-restricted processing pathway, exogenous Ag can enter the class I pathway of APCs if it is present in high concentrations, is processed or degraded extracellularly, or is associated with cell debris (25, 26). Recently published data suggest that macrophages can phagocytose and process fragments of apoptotic cells for MHC...
class I-restricted presentation (27). DC populations used in these experiments are actively phagocytic (our unpublished data) and could take up and present tumor Ags through a similar mechanism. In addition, it is possible that close association of tumor cells with DCs could facilitate the transfer of tumor-specific peptides chaperoned by heat shock proteins (7).

Our studies demonstrate that PEG-mediated fusion is not required for immunogenicity. However, generation of an immune response does require that DCs and tumor cells be tightly associated before injection, as much mock-fused cocultured cells were immunogenic, but the same number and ratio of DCs and tumor cells injected together without in vitro coculture were not (Table I, Fig. 28). These results are in agreement with those of Guo et al. (20) and Gong et al. (21), who similarly compared the immunogenicity of APC-tumor cell fusions with cocultured APCs and tumor cells. However, the immunogenicity of cellular vaccines consisting of the unselected products of DC-tumor cell fusions or DCs and tumor cells that have been cocultured but not fused was not directly evaluated (21). It is also notable that immunizations with DCs and tumor cells that were cocultured in chambers separated by a permeable membrane were not immunogenic (Table I, Fig. 2). Although this suggests that immunogenicity was not mediated by the transfer of soluble factors, this possibility cannot be ruled out because functional transfer of Ag or other factors may depend on localized release and uptake and may be more efficient when cells are closely associated. In this way, intimately associated DCs and tumor cells could communicate in an “autocrine” fashion.

DCs can be found within tumors in vivo and in some instances DC infiltration has been associated with improved prognosis. This association may represent a “natural” correlate of the immunogenicity we observe following injection of ex vivo-associated DCs and tumor cells. Clearly, DC infiltration into tumors is not always sufficient for tumor rejection. Recent studies suggest that at least in some instances, mature DCs from tumor-bearing hosts can have defects in APC function (28). In addition, human cancer cells can release soluble factors that can inhibit the maturation of DCs (29). The immunization strategy we describe here could circumvent tumor-induced APC dysfunction by utilizing functional DCs derived from DC precursors in vitro. In this regard, recent studies suggest that DCs grown from precursors obtained from tumor-bearing hosts can be effective Ag delivery vehicles for tumor immunotherapy (30). Whether or not injection of functional, in vitro-derived DCs directly into tumors in vivo will induce a tumor-specific immune response has yet to be determined. The immunogenicity of DC-tumor cell conjugate vaccines supports the feasibility of this approach.

DC-tumor cell conjugate vaccines have several features that suggest potential translational applications for the immunotherapy of human tumors. Human DCs, phenotypically and functionally similar to the murine DCs used in our studies, can be obtained readily by in vitro culture of peripheral blood-derived precursors (18). Preparation of the DC-tumor cell immunogen is rapid and does not require additional selection of stable fusion products, minimizing the interval between tumor excision and immunization and suggesting a potentially broad application to multiple tumor types. DC-tumor cell association occurs with high efficiency, and injected cells are irradiated and nonproliferating. DC-tumor cell immunization has the potential to stimulate immunity against multiple tumor Ags and could induce synergetic protection through CD4+ and CD8+ T cell-mediated immune responses. Because the tumor cell is the source of Ag, immunizations would not depend on the prior identification of unique or “shared” tumor Ags and would not be limited to individuals expressing a particular corresponding MHC allele. Furthermore, because the immunization is patient specific, it could stimulate immunity against uniquely expressed tumor Ags that may be an important component of an effective antitumor response.

Acknowledgment

We thank Robert Lakomy for his helpful assistance with flow cytometry.

References


Downloaded from http://www.jimmunol.org by guest on September 15, 2017