Migration of Human Dendritic Cells after Injection in Patients with Metastatic Malignancies

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Abstract

Present clinical studies of active immunotherapy for malignancies using dendritic cells (DCs) require elucidation of the sites where DCs localize after injection. We evaluated the pattern of distribution of in vitro-generated, antigen-loaded, human DCs labeled with indium-111 oxyquinoline after i.v., s.c., and intradermal injection. Whereas the DCs injected i.v. localized in the lungs and then redistributed to the liver, spleen, and bone marrow, they were not detected in lymph nodes or tumors. A small percentage of DCs injected intradermally migrated rapidly to the regional lymphatics in some individuals. No lymph node activity was detected after s.c. injection. Our results demonstrate that DC distribution to sites of lymphoid tissue is dramatically affected by the mode of administration.

Introduction

Active immunotherapy strategies using tumor antigen-loaded DCs depend on DC interactions with T cells. The best route of DC administration for ensuring migration to T cell-rich sites is uncertain, in part, because the sites where human DCs localize after injection are unknown. Although bone marrow-derived DCs or their precursors circulate in the peripheral blood to most tissues, acquire antigen, and subsequently migrate to regional lymph nodes (1, 2), it remains uncertain whether DCs (generated in vitro under the influence of cytokines such as GM-CSF and IL-4; Refs. 3–5) exhibit equivalent migratory capacity. Murine (6–8) and primate (9) studies have demonstrated that i.v.-administered DCs initially localize in the lungs and then redistribute to the liver, spleen, and bone marrow, but apparently not to the lymph nodes or tumors. A small percentage of DCs injected s.c. appear in the lungs, followed several hours later by accumulation in the primate. These results suggest that the route of DC administration influences the ability of DCs to seed metastatic sites in the primate.

Patients and Methods

Patients. The patients (n = 7) consisted of a subset of those enrolled on a Phase I study of active immunotherapy with CEA RNA-loaded DCs. All patients were required to have a metastatic cancer-expressing CEA (breast (1), lung (4), and colon (2)) and evidence of a recall antigen response on skin testing with either Tetanus, Mumps, Candida, or Histoplasma. Potential candidates for the study were excluded if they had received chemotherapy, radiation therapy, immunotherapy, or immunosuppressive medications within the prior 4 weeks. Candidates for intradermal injections were excluded if they had had a lymph node dissection or known lymphatic obstruction in the bed draining the injection site. Study subjects provided signed informed consent that fulfilled Institutional Review Board guidelines before completing the screening procedures.

Generation of DCs. The patients underwent an unimmobilized peripheral blood leukapheresis, consisting of a 3–4-h processing of 8–12 liters of blood with each collection containing a minimum of 1.5 × 10^9 nucleated cells/kg recipient weight. PBMCs were separated from the leukapheresis product, resuspended in AIM V media (Life Technologies, Grand Island, NY) at 6 × 10^6 cells/ml, and incubated in T225 tissue culture flasks (60 ml/flask) for 2 h in 5% CO\textsubscript{2} at 37°C. The nonadherent cells were gently resuspended by rocking the flasks and removed. The adherent cells were cultured in AIM V media containing GM-CSF (800 units/ml) and IL-4 (500 units/ml; kind gifts of Mary-Ellen Ryback, Schering-Plough Research Institute, Kenilworth, NJ) for 7 days to generate a cell population enriched for DCs. After 7 days, the cultured cells were harvested by vigorous washing and the addition of cell dissociation buffer (Life Technologies). The DCs were depleted of bystander lymphocytes and natural killer cells by use of an antibody mixture containing anti-CD3, -CD16, -CD19, -CD56, magnetic colloid, and a magnetic column (Stem Cell Technologies, Vancouver, British Columbia). The cells not retained in the column were >90% DCs by morphology. The DCs were coincubated with CEA RNA (produced as described previously; Ref. 10) for 2–4 h and washed twice in normal saline.

In-111 Labeling. The CEA RNA-loaded DCs were labeled with In-111 as follows: for patients receiving a dose of 100 × 10^6 DCs i.v., the DCs were resuspended in 500 μCi In-111 (Nycomed-Amersham, Chicago, IL) in a total volume of 1 ml. This was calculated to result in ~200 μCi of radioactivity administered with the DCs, an amount felt to be safe based on calculations of radiation exposure of the lungs, liver, and spleen. Incubation was carried out at room temperature for 1 h, after which the cells were washed three times, and the labeling efficiency was determined by measuring the amount of radioactive contained within the cellular fraction compared with the supernatant. The DCs were resuspended in a volume of 15–30 ml of normal saline for i.v. injection. For patients receiving DCs intradermally or s.c., 1 × 10^7 DCs were similarly labeled with the In-111, except that only 10 μCi of radioactivity was used to limit the radiation exposure of the skin. These DCs were resuspended in 1 ml of normal saline for injection.

Immunofluorescence Staining and Fluorescence-activated Cell Sorting Analysis. To demonstrate that the phenotype of the DC was not altered by the labeling procedure, immunofluorescence staining with the monoclonal antibodies anti-CD14-FITC, anti-CD86-PE, and anti-HLA-DR-PerCp (Becton Dickinson, San Jose, CA) and fluorescence-activated cell sorting analysis were performed on the DCs before and after labeling with the In-111, as previously described (5).

Allogeneic MLR. The allogeneic MLR was performed on the DC before and after labeling with the In-111 to determine whether the function of the DC was altered by the labeling. Allogeneic responder PBMCs (1.5 × 10^5) obtained from healthy donors were cultured in RPMI 1640 supplemented with 10% fetal bovine serum or 10% human AB serum in 96-well U-bottomed tissue culture plates. Irradiated (3500 rads) DC preparations (or the patient’s PBMCs used as controls) were added in graded doses of 150–15000 cells/well in a total volume of 200 μl. Cell proliferation after 96 h was quantified by adding 1 μCi of [methyl 3H]thymidine (NEN-DuPont, Boston, MA) to each well. After 16 h, the cells were harvested onto filters, and radioactivity was measured.
Table 1 Localization of i.v.-administered In-111-labeled DCs

<table>
<thead>
<tr>
<th>Time</th>
<th>Right lung</th>
<th>Left lung</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>maximum activity (%)</td>
<td>maximum activity (%)</td>
<td>maximum activity (%)</td>
<td>maximum activity (%)</td>
</tr>
<tr>
<td>1 min</td>
<td>100</td>
<td>100</td>
<td>4 ± 5</td>
<td>3 ± 0</td>
</tr>
<tr>
<td>1 hr</td>
<td>74 ± 19</td>
<td>77 ± 13</td>
<td>30 ± 9</td>
<td>36 ± 11</td>
</tr>
<tr>
<td>6 hr</td>
<td>25 ± 9</td>
<td>28 ± 5</td>
<td>86 ± 9</td>
<td>87 ± 9</td>
</tr>
<tr>
<td>24 hr</td>
<td>11 ± 5</td>
<td>13 ± 2</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

The ideal vaccination protocol for inducing protective immune responses is not established. Animal models have suggested that...
peptide-loaded DCs administered i.v. are more effective than those administered s.c. in protecting against a tumor or infectious challenge (11, 12). Nonetheless, it is not known where the i.v. administered DC contact the T cells during induction of the immune response. Fossum (8) observed that i.v.-injected DCs localized in the liver and spleen of rats. From the liver, some of the cells migrated to celiac lymph nodes. DCs injected into the footpad remained primarily localized, though a few migrated to the popliteal nodes. Similar observations were made in mice (7) and chimpanzees (9), except that either trace (7) or no (8) lymph node migration was detected after i.v. injections. Our results are similar to the animal studies in that the patient DCs localized first to the lungs, and then the liver, spleen, and bone marrow after i.v. injection, whereas a small amount of lymph node transit (0.1–0.4% of the injected activity) was observed for intradermal injections in some patients. Although the number of DCs that must reach lymphatic tissue for an effective immune response in humans is unknown, Porgador et al. (13) showed in a murine model that i.v. administration of as few as 9 x 10^3 DCs pulsed with tumor peptides had a measurable antitumor effect. If a similar potency of DCs applies to humans, the number of DCs we observed to migrate from intradermal sites may be adequate to induce an antitumor response. Despite the small amount of activity appearing in regional lymphatics, 20–30% of the activity was lost from the injection sites indicating that the DCs may migrate to other sites, bypassing the regional lymphatics, but we could not identify these sites. Although it is possible that the limits of resolution of the In-111 imaging technique do not permit detection of very small amounts of DC-associated activity in lymph nodes, it is clear that most of the activity in the first 24 h is localized to the liver, spleen, and bone marrow after i.v. injection, and in the local site after intraderal or s.c. injection. Because the rate of change in the level of activity at each site was leveling off by 48 h, we do not believe that further monitoring would have detected a more selective localization of DCs in tumors or lymph nodes. It is also clear that tumors, even those in the lungs, are not infiltrated with the i.v. injected DCs. We suspect that this is due to the fact that the lung tumors are supplied primarily by bronchial arteries (derived from the aorta) and not the pulmonary arteries (14). The bronchial vasculature does not possess the narrow channels found in the pulmonary vasculature, liver, and spleen in which injected cells are likely to lodge (15), and thus tumors supplied by bronchial arteries are no more likely to have a significant exposure to i.v.-injected DCs than organs such as the kidney or brain.

Our data indicate that DC trafficking is markedly dependent on their mode of delivery. s.c. administration seems to be ineffective in causing DC migration to regional lymphatics. i.v. administration results in DC migration to the spleen, whereas intradermal administration leads to regional transit in some patients. Which site of T cell contact will lead to greater antigen-specific immune responses is uncertain. Murine studies indicating that migration patterns of DCs may determine whether Th1 or Th2 responses are induced (16) underscore the need to compare immunological responses induced with each route of injection in order that the ideal strategy be used in clinical trials. Whether the pattern of localization can be altered by the use of other cytokines or agents to modulate adhesion molecules remains to be studied.

Acknowledgments

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References


Table 2. Localization of intradermally or s.c.-administered In-111-labeled DCs

<table>
<thead>
<tr>
<th>Time</th>
<th>s.c. injection site maximum activity (%)</th>
<th>Intradermal injection site maximum activity (%)</th>
<th>Inguinal LN* draining ID site maximum activity (%)</th>
<th>Inguinal LN draining ID site injection site activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>100</td>
<td>100</td>
<td>56 ± 5</td>
<td>0.08</td>
</tr>
<tr>
<td>6 hr</td>
<td>93 ± 3</td>
<td>89 ± 2</td>
<td>75 ± 4</td>
<td>0.19</td>
</tr>
<tr>
<td>24 hr</td>
<td>82 ± 4</td>
<td>77 ± 2</td>
<td>95 ± 7</td>
<td>0.37</td>
</tr>
<tr>
<td>48 hr</td>
<td>70 ± 4</td>
<td>65 ± 4</td>
<td>92 ± 3</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* LN, lymph nodes; ID, intradermal.
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