Maturation and Trafficking of Monocyte-Derived Dendritic Cells in Monkeys: Implications for Dendritic Cell-Based Vaccines


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Maturation and Trafficking of Monocyte-Derived Dendritic Cells in Monkeys: Implications for Dendritic Cell-Based Vaccines


Human dendritic cells (DC) have polarized responses to chemokines as a function of maturation state, but the effect of maturation on DC trafficking in vivo is not known. We have addressed this question in a highly relevant rhesus macaque model. We demonstrate that immature and CD40 ligand-matured monocyte-derived DC have characteristic phenotypic and functional differences in vitro. In particular, immature DC express CC chemokine receptor 5 (CCR5) and migrate in response to macrophage inflammatory protein-1α (MIP-1α), whereas mature DC switch expression to CCR7 and respond exclusively to MIP-3β and 6Ckine. Mature DC transduced to express a marker gene localized to lymph nodes after intradermal injection, constituting 1.5% of lymph node DC. In contrast, cutaneous DC transfected in situ via gene gun were detected in the draining lymph node at a 20-fold lower frequency. Unexpectedly, the state of maturation at the time of injection had no influence on the proportion of DC that localized to draining lymph nodes, as labeled immature and mature DC were detected in equal numbers. Immature DC that trafficked to lymph nodes underwent a significant up-regulation of CD86 expression indicative of spontaneous maturation. Moreover, immature DC exited completely from the dermis within 36 h of injection, whereas mature DC persisted in large numbers associated with a marked inflammatory infiltrate. We conclude that in vitro maturation is not a requirement for effective migration of DC in vivo and suggest that administration of Ag-loaded immature DC that undergo natural maturation following injection may be preferred for DC-based immunotherapy.


Materials and Methods

Animals

Normal adult rhesus macaques (Macaca mulatta) were used in this study. Animals were housed and all in vivo experiments were performed at the University of Pittsburgh, Pittsburgh, PA 15261. E-mail address: smbb@pitt.edu

1 Abbreviations used in this paper: DC, dendritic cells; CCR, CC chemokine receptor; CD40L, CD40 ligand; MIP, macrophage inflammatory protein; i.d., intradermal; DiD, 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate; EGFP, enhanced green fluorescence protein.

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Infectious Disease Primate Research Facility, University of Pittsburgh (Pittsburgh, PA), using protocols approved by the institutional animal care and use committee.

**Media and reagents**

The medium used to culture DC was RPMI 1640 medium supplemented with 10% FCS (BioWhittaker, Walkersville, MD), l-glutamine, sodium pyruvate, nonessential amino acids, penicillin-streptomycin, 10 mM HEPES buffer (Life Technologies, Grand Island, NY), and 2-ME (Sigma, St. Louis, MO). Recombinant human GM-CSF and IL-4 were provided by Schering-Plough (Kenilworth, NJ), and trimeric human CD40L was provided by Immunex (Seattle, WA). Recombinant human MIP-1α, 6CinKine (BioSource, Camarillo, CA), and MIP-3β (R&D Systems, Minneapolis, MN) were used in chemotaxis assays. QD Green BSA was a gift from Mingjie Zhou (Molecular Probes, Eugene, OR). The lipophilic carboxycyanine dye 1,1'-dioctadecyl-3,3',3',3'-tetramethylindod-carbocyanine perchlorate (DiD; excitation/emission spectra = 644 nm/663 nm) was used in labeling studies was purchased from Molecular Probes.

**DC culture**

PBMC were isolated from heparinized blood by centrifugation through sodium diatrizoate and Ficoll (Sigma), and CD14+ cells were positively selected using Ab-coated microbeads and magnetic separation (Miltenyi Biotec, Auburn, CA). The purity of CD14+ cells using this technique was consistently >90%. Cells were cultured at a density of 3 × 10^6 cells/ml in six-well plates. GM-CSF (1000 U/ml) and IL-4 (1000 U/ml) were added at the initiation of culture, and media and cytokines were replenished at regular intervals for 7 days, depending on the experiments. CD40L (3 μg/ml) was added on day 5 of culture for 24–48 h to mature cells, and cells were harvested following incubation with 10 mM EDTA.

**Flow cytometric analysis**

DC were stained for flow cytometric analysis as previously described (13). Cross-reactive mAb specific for human HLA-A, -B, and -C (clone B9.12.1); CD83 (HB15A; both from Coulter-Immunootech, Miami, FL); HLA-DR (L243); CD14 (m699); CD80 (L370.4; all from Becton Dickin-son, San Jose, CA); CD86 (FUN-1; PharMingen, San Diego, CA); CD40 (EA-5; Ancell, Bayport, MN); and CCR5 (FAB182; gift from Frank Kron-tari, R&D Systems) were used as conjugates with FITC or PE. In some experiments biotinylated CD86 was used with streptavidin-Cy5 (Amer- sham, Arlington Heights, IL). The CCR7 mAb was a gift from Lijun Wu (LeukoSite, Cambridge, MA) and was used in association with a goat anti-mouse secondary Ab (BioSource). Analyses related to the characterization of immature and mature DC were performed on a FACS Calibur flow cytomter (Becton Dickinson) using CellQuest software. For analyses of DC and lymph node suspensions in trafficking studies, 1.5 × 10^6 events were acquired in triplicate on a FACS Vantage flow cytomter (Becton Dickinson), using a second argon laser emitting at 647 nm to excite DiD fluorescence. Data were acquired and analyzed using LYSIS II and WinList software, respectively. Fluorescence and side scatter measurements for in vivo data are shown in four-decade log scales.

**Assessment of Ag uptake**

For analysis of macropinocytosis by DC we used QD Green BSA, a self-quenched dye conjugate of BSA. Degradation of protein in this reagent results in dequenching and elaboration of green fluorescence (14). DC were incubated with 10 μg/ml QD Green BSA at 4 or 37°C for 30 min with and without prior incubation with 10 μg/ml cytochalan D (Sigma) followed by labeling with mAb to CD86. Fluorescence in this assay is indicative of uptake and proteolytic cleavage of BSA.

**Analysis of cytokine production**

Supernatants were collected from cultures on days 4 and 7, with and without addition of CD40L (3 μg/ml) on day 5, and stored at −70°C until assayed. IL-12 (p40 and 70 forms) and TNF-α were detected using ELISA kits specific for rhesus monkey (BioSource) according to the manufacturer’s instructions.

**Chemotaxis assays**

Cell migration was measured using a 96-well chemotaxis chamber with a 5-μm pore polycarbonate membrane (Neuro Probe, Gaithersburg, MD). Thrombin microtiter of chemokine in RPMI 1640 and QD Green BSA, and 10 mM HEPES medium was added to appropriate wells, which were then covered by the membrane. Twenty-five thousand cells at a density of 1 × 10^6 cells/ml in the same medium were placed on the membrane. Plates were incubated for 90 min at 37°C in a humidified incubator, and cells remaining on the topside of the membrane were then removed by gentle washing with PBS and wiping. Migrated cells that had attached to the underside of the membrane were fixed in 2.5% glutaraldehyde and stained with 0.1% toluidine blue (Fisher Scientific, Pittsburgh, PA). To quantify migration, cells in four nonoverlapping areas were counted under ×400 magnification and summed. Results are expressed as the mean ± SEM for duplicate wells.

**Transduction of monkey DC using recombinant adenoaviruses encoding a marker gene**

DC were transduced with a replication-defective recombinant adenoviral vector expressing enhanced green fluorescence protein (EGFP) under the control of the CMV immediate-early promoter/enhancer (15), which was provided by Andrea Garzon (University of Pittsburgh). Briefly, virus was added at a multiplicity of infection of 100 directly to wells containing day 4 cells and incubated for 1 h at room temperature. Medium was then replenished, and cells were matured with addition of CD40L for 24 h.

**DC trafficking in vivo**

Immature day 4 or mature CD40L-treated day 7 DC were labeled with DiD as previously described (12) or were transduced with recombinant adenoaviruses and resuspended in 400 μl of PBS for injection. Between 2.7 × 10^5 and 5.2 × 10^5 DC were injected i.d. into anesthetized animals from which cells were derived in a region lateral to the proximal inguinal lymph node chain. In some experiments EGFP gene was administered via gene gun to skin before injection of labeled DC. Animals were anesthetized 36 h later, and inguinal and axillary lymph nodes and skin were biopsied. Lymph node tissues were disrupted using digestion with collagenase D as previously described (16). Unseparated lymph node cell suspensions were labeled with mAb to CD83 and CD86 before flow cytometric analysis. Skin biopsies were treated with 4% parafomaldehyde and 30% sucrose infusion before freezing in isopentane. Sections were cut and stained with hematoxylin and eosin or were examined directly for fluorescent cells as previously described (12).

**DNA-based immunization**

Genetic immunizations were performed by biolistic delivery as previously described (17) using the pEGFP-C2 plasmid (Clontech, Palo Alto, CA), which contains the EGFP gene under the control of the CMV promoter. Animals received a total of four shots given to two overlapping regions of shaved skin in the inguinal region. In all cases the gene gun was applied before and at least 0.5 in. away from injection of cells. In experiments in which the gene gun was applied, injected DC were labeled with DiD so as to distinguish in situ transfected cells (green) from injected DC (red).

**Results**

**Monkey DC undergo phenotypic and functional maturation upon treatment with CD40L.**

To establish the monkey DC system we performed extensive phenotypic and functional studies in vitro. DC cultured from CD14+ peripheral blood monocytes of rhesus macaques had characteristic dendritic morphology (data not shown), similar to that of human CD14-derived DC (18) and DC generated by us and others from PBMC of the chimpanzee and macaque (13, 19). DC cultured for 4 days in GM-CSF and IL-4 retained expression of CD14, but were negative for the mature DC marker CD83 (Fig. 1). The day 4 DC expressed moderate levels of costimulatory molecules CD80, CD86, and CD40 and high levels of MHC class I and class II (Fig. 1). In contrast, monkey DC treated by ligation of CD40, which is known to induce rapid maturation of DC in the human system (20, 21), uniformly expressed CD83 and high levels of costimulatory molecules CD80, CD86, and CD40 (Fig. 1). Expression of MHC class I and class II was unaltered, whereas expression of CD14 was down-regulated. Cells cultured for 7 days without CD40 ligation had an intermediate phenotype (Fig. 1).

To examine functional changes associated with CD40L treatment we tested the capacity of DC to exhibit macropinocytosis and secrete proinflammatory cytokines, properties of immature and mature DC, respectively (21, 22). DC cultured for 4 days rapidly internalized and cleaved QD Green BSA when incubated at 37°C,
but not at 4°C (Fig. 2). This required actin polymerization and, hence, was an active process, as treatment with cytochalasin D substantially inhibited uptake (22). In contrast, DQ Green BSA fluorescence was almost completely absent at 37°C when CD40L-matured day 7 cells were used (Fig. 2). Cells cultured for 7 days in the absence of CD40L had an intermediate function. The loss of ability to internalize and cleave protein upon maturation was correlated with increased expression of CD86, as can be seen by the small proportion of day 4 DC that did not process DQ Green BSA and expressed high levels of CD86 (Fig. 2). Hence, in this system CD86 up-regulation is a reliable indicator of functional maturation.

In cytokine release assays, day 4 and day 7 DC secreted negligible levels of IL-12 and TNF-α, whereas CD40L-treated day 7 DC markedly up-regulated secretion of these cytokines (Table I). Based on these results, in subsequent chemotaxis and trafficking experiments we classified cells harvested on day 4 of culture as immature DC and cells harvested on day 7 after CD40L treatment as mature DC.

Immature and mature monkey DC have distinct patterns of chemokine receptor expression and chemotactic responses

We next determined the chemokine receptor expression and chemotactic responsiveness of monkey DC at different stages of maturation. Chemokine receptor expression of human DC is highly polarized, with immature DC expressing receptors for inflammatory chemokines and switching expression to receptors for constitutive chemokines upon maturation (2–5, 23). Consistent with the

Table I. CD40L-treated monkey DC secrete high levels of IL-12 and TNF-α

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>IL-12</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4 DC</td>
<td>59.2</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Day 7 DC</td>
<td>18.6</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Day 7 DC/CD40L</td>
<td>33,142.7</td>
<td>455.0</td>
</tr>
</tbody>
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*Means of duplicate samples. Standard errors were always <10% of the means. *p70 and free p40 subunits. Results are representative of three experiments.

FIGURE 1. CD40L induces maturation of monkey monocyte-derived DC. DC grown for 4 or 7 days in GM-CSF and IL-4, with and without treatment with CD40L on day 5, were stained with cross-reactive mAb and analyzed by flow cytometry. CD40L induces expression of CD83, loss of CD14, and increased expression of CD80, CD86, and CD40. Expression of MHC classes I and II remained high and unchanged by CD40L treatment.

FIGURE 2. Day 7 monkey DC treated with CD40L lose the capacity to internalize and process soluble protein, correlated with an increase in expression of CD86. a, Green fluorescence of DC following incubation with 10 μg/ml DQ Green BSA for 30 min. Fluorescence results from proteolytic cleavage of the protein. Incubations were performed at 4 or 37°C with and without pretreatment with cytochalasin D (CCD). b, Expression of CD86 vs green fluorescence by DC incubated with 10 μg/ml DQ Green BSA for 30 min at 37°C.
human DC literature, immature monkey DC expressed low levels of CCR5, but not CCR7. Conversely, CD40L-matured DC were negative for expression of CCR5, but expressed CCR7 at high levels (Fig. 3). When monkey DC were tested in chemotaxis assays, day 4 immature DC migrated in response to CCR5 ligand MIP-1α, but not at all to the CCR7 ligands 6Ckine and MIP-3β. Upon maturation, chemotactic responses were completely switched to MIP-3β and 6Ckine (Fig. 3). The weaker response of immature DC to chemokine correlated with the low level expression of CCR5 on these cells. These data indicate that in vitro chemotactic responses of monkey monocyte-derived DC are tightly regulated as a function of maturation.
Mature monkey DC transduced with EGFP ex vivo traffic to lymph nodes in greater numbers than cutaneous DC transfected in situ with EGFP

To test the capacity of in vitro-derived DC to traffic to lymph nodes, we first transduced DC ex vivo with a gene expressing the cytoplasmic Ag EGFP using recombinant adenovirus and then matured the cells with CD40L. Transduced cells were injected i.d. into the inguinal region, and the draining lymph node was excised 36 h later. Approximately 99% of DC expressed EGFP before injection, and a proportion of these cells could subsequently be identified in the draining lymph node (Fig. 4). No EGFP fluorescence was detected in control axillary lymph nodes (data not shown). When lymph node cells were stained for expression of CD83 and CD86 immediately before injection, a proportion of CD83<sup>+</sup> cells could be detected in draining lymph nodes, cells constituting ~1% of the total lymph node was identified, representing interdigitating DC (24, 25). The EGFP<sup>+</sup> DC that localized to lymph nodes were also CD83<sup>−</sup>CD86<sup>−</sup>, as expected from our in vitro analyses of mature DC, and represented 1.5% of total lymph node DC (Fig. 4). A proportion of CD83<sup>−</sup> cells was also identified within the region describing EGFP<sup>+</sup> cells in the lymph node. This may reflect endogenous cells that have a level of autofluorescence overlapping that of EGFP<sup>+</sup> cells. To place this result in the context of other DC-based vaccine protocols, in additional experiments we administered EGFP gene via gene gun to the inguinal skin of monkeys and excised lymph nodes 36 h later. Skin DC transfected in situ with EGFP gene were not detected in the draining lymph nodes of two animals using flow cytometric analysis. This is not surprising, as in previous murine studies fluorescence microscopy has been required to identify the small number of transfected cells in lymph nodes (17, 26). When draining lymph nodes from four other animals were sectioned in their entirety and examined, occasional EGFP<sup>+</sup> cells were identified (data not shown). Whereas few EGFP<sup>+</sup> cells could be detected in draining lymph nodes, cells containing gold particles and expressing EGFP could be identified in the superficial dermis (Fig. 5). An acute inflammatory response was present in the epidermis as a result of gene gun bombardment (Fig. 5).

Migration of monkey DC to lymph nodes is not dependent on prior in vitro maturation

The above experiments indicated that mature Ag-stimulated monkey DC could efficiently traffic to lymph nodes after i.d. injection, as was predicted from our in vitro chemotaxis assays. We next wanted to directly compare the migration of immature and mature DC in vivo. We elected to switch labeling techniques from the adenovirus system to eliminate any potential for inadvertent maturation of immature DC before injection. We therefore used an inert lipophilic membrane dye to label cells as in our previous studies (12). In separate experiments, DiD-labeled immature and mature DC were injected i.d. into the inguinal region of donor monkeys, and the draining inguinal lymph nodes were excised 36 h later and analyzed by flow cytometry. Labeled DC were stained for expression of CD83 and CD86 immediately before injection to confirm the maturation state of cells. Immature cultured DC were CD83<sup>−</sup>CD86<sup>−</sup>, as expected from our previous analyses (Fig. 6). In contrast, mature cultured DC were CD83<sup>−</sup>CD86<sup>bright</sup>, expressing...
significantly higher levels of CD86 than immature DC (Fig. 6). Following injection, DiD$^+$ cells were identified in the draining lymph node of animals receiving both immature and mature DC (Fig. 6). No DiD$^+$ cells could be identified in control axillary lymph nodes (data not shown). The DiD$^+$ cells constituted 1% of total lymph node DC regardless of the in vitro maturation state (Fig. 6). The proportion of injected cells that were identified in the lymph node at 36 h ranged from 0.07 to 0.12% over five experiments.

Evidence for regulation of DC maturation in vivo

From our in vitro data we know that immature DC do not migrate in response to constitutive lymphoid chemokines MIP3 and 6Ckine, yet the in vivo trafficking results clearly indicate migration of these cells to lymph nodes in significant numbers. One explanation for this difference is that upon injection the immature DC undergo spontaneous maturation. To evaluate this possibility we compared expression of CD86 on immature DC before injection with these cells following trafficking to lymph nodes. CD86 up-regulation is a valid marker of maturation, as we have shown in experiments in vitro (Fig. 2). The mean fluorescence intensity of CD86 on immature DC that localized to lymph nodes was 3.6-fold higher than that of immature DC before injection, suggestive of in vivo maturation (Fig. 7). Surprisingly, CD86 expression on mature DC that had localized in lymph nodes was, on the average, 3.9 times lower than that of the same cells before injection. As a result, expression of CD86 on DC that localized to lymph nodes was essentially the same regardless of the maturation state at the time of injection (Fig. 7).

Complete migration of immature, but not mature, DC from skin

To determine the relative efficiency of migration of immature and mature DC from the dermal injection site, we examined skin biopsied 36 h after injection of cells. Injection of immature DC resulted in a minor localized acute inflammatory response. No fluorescently labeled cells could be identified at this time, suggesting complete migration of immature DC from the injection site (Fig. 8). In marked contrast, a severe acute inflammatory infiltrate was present at the site of injection in two of three animals that received injections of mature DC. A large number of fluorescently labeled mature DC were detected in the dermis at 36 h in these animals (Fig. 8). The accumulation of DC was not dependent on labeling with DiD, as mature DC that expressed EGFP by adenoviral transduction were also identified in large numbers in a separate experiment (data not shown).

Discussion

The purpose of this study was to determine in a primate model the extent to which in vitro maturation of DC influences the capacity of DC to traffic in vivo. We were able to reproducibly generate immature and mature DC from purified CD14$^+$ monocytes from monkey blood using the cytokines GM-CSF and IL-4 with CD40L as a maturation factor (20, 21). Immature DC were characterized as CD83$^-$CD86$^+$ cells that could rapidly pinocytose and process soluble
protein but produce negligible quantities of proinflammatory cytokines. Mature DC were characterized as CD83<sup>+</sup>CD86<sup>+</sup>high cells that were ineffective at processing soluble Ag but produced large quantities of IL-12 and TNF-α. These are recognized properties of immature and mature DC as defined in the human system (1). Monkey DC had the expected chemokine receptor profiles, switching from expression of CCR5 on immature cells to CCR7 following maturation with CD40L (2–4, 23). Accordingly, immature DC migrated exclusively in response to the inflammatory chemokine MIP-1α, whereas mature DC migrated to constitutive chemokines 6Ckine and MIP-3β that are important in attracting DC to T cell areas of lymph nodes (5–7). The clear in vitro similarities between monocyte-derived DC in the human and monkey suggest that conclusions drawn from in vivo experiments using this model can safely be extrapolated to the human system.

For the in vivo trafficking experiments, we focused on flow cytometric methods to accurately determine the number and phenotype of DC that migrated to lymph nodes following labeling and injection. Unexpectedly, we found that immature and CD40L matured DC trafficked to lymph nodes with similar efficiency despite the polarized chemokine receptor expression and in vitro chemotactic responses of these cells. In several experiments using two different labeling techniques, the proportion of lymph node DC that were derived from the injected population was ~1% at 36 h postinjection regardless of the state of maturation at the time of injection. Given this relatively large number, it is likely that Ag-loaded DC will readily contact and stimulate Ag-specific T cells that migrate through the paracortex, indicating that DC-based vaccination should be effective at stimulating immune responses in large animals and humans. Using histological techniques in a similar chimpanzee model, we have previously demonstrated that injected DC localize to the lymph node paracortex, with a peak migration occurring from 24–48 h post injection (12). Injected DC intimately associate with T cells in this region and maintain high levels of HLA-DR, CD40, and CD86 expression similar to interdigitating DC (12). In preliminary studies in monkeys we noted that immature and mature DC both localized to T cell-rich areas of lymph nodes when administered together in equal numbers, using different fluorochromes to track cells (data not shown). From these analyses we can conclude that labeled DC detected in tissues by flow cytometry have actively trafficked to lymph nodes and taken up residence as interdigitating DC.

Our findings are consistent with data from the mouse using purified splenic DC of the myeloid lineage (CD8<sup>+</sup>), which are analogous to human monocyte-derived DC. These cells traffic to lymph nodes following s.c. injection in the absence of any in vitro maturation stimulus, representing about 1% of total lymph node DC (27). A recent report in the murine system indicates that immature bone marrow-derived DC migrate poorly to lymph nodes following s.c. injection, relative to migration of GM-CSF transfected immature DC (28). Interestingly, GM-CSF transfection did not induce maturation of DC in this system, based on phenotypic and functional analysis (28). When the migration of immature and CD40L matured DC was compared in the same system, injected DC that trafficked to lymph nodes constituted from 1–2% of lymph node DC 48 h after s.c. injection regardless of maturation state (11).

Two potential explanations exist for the trafficking of immature DC to lymph nodes. The first is that immature DC respond to as yet unknown chemokines that do not use the CCR7 receptor. This is unlikely, as 6Ckine, which binds CCR7, appears to be required for DC homing to lymph nodes. Mice lacking expression of 6Ckine have a paucity of DC in lymph nodes (7), and administration of Ab to 6Ckine blocks the migration of mature DC to lymph nodes when injected s.c. into mice (5). A more likely explanation is that immature DC undergo spontaneous maturation upon injection into skin. Consistent with this hypothesis was the finding that labeled DC that trafficked to lymph nodes had a statistically significant increase in CD86 expression compared with cultured immature DC, which is suggestive of functional DC maturation on the basis of our in vitro experiments and other reports in mice (29). A mild inflammatory response was present in the skin at the site of injection at 36 h, and hence injected DC would be exposed to proinflammatory cytokines that induce maturation (30, 31). Interestingly, while CD86 expression was up-regulated on immature DC following localization to lymph nodes, a decrease in expression of this costimulatory molecule was noted on mature DC that migrated to lymph nodes, although this change was not statistically significant. Moreover, the relative changes in CD86 expression of injected immature and mature DC resulted in an almost identical expression of CD86 by these cells in lymph nodes even though the data were collected from different animals in different experiments. Removal of DC from in vitro exposure to GM-CSF and IL-4 is not likely to be responsible for the decrease in CD86 expression by mature DC, given the up-regulation of CD86 by immature cells subjected to the same conditions. However, it is possible that withdrawing cells from high dose exogenous CD40L resulted in modulation of CD86 expression. The data raise the possibility that homeostatic mechanisms exist in vivo that regulate the degree of activation of exogenously supplied DC upon localization to the lymph node.

A second unexpected finding from the in vivo studies was that immature DC migrated completely from skin following i.d. injection in monkeys, whereas mature DC tended to remain in the dermis. Our previous studies in the chimpanzee also demonstrated that in vitro-derived DC, labeled with DiD but administered without Ag or prior maturation, migrated away from an s.c. injection site within 48 h of injection (12). Foussom (32) reported that purified lymph DC injected into footpads of mice mostly were retained at the site of injection. Lymph DC represent a maturing DC population (33), and hence these findings may be consistent with our results. More recently, Ag-stimulated blood-derived human DC used therapeutically in human cancer patients were only partially cleared from an i.d. injection site and apparently not at all following s.c. injection (34). Labeur et al. (11) also found that the majority of mouse DC remained at the site of s.c. injection regardless of the in vitro maturation state before administration. The differences between our report and others may be due in part to the types of labels used and the different species studied. However, our findings raise the question of whether there is a fundamental difference between the capacity of immature and mature DC to be cleared from a skin injection site. Mature DC produce large quantities of proinflammatory cytokines and probably induced the severe inflammatory response observed in the dermis. Therefore, while a mild inflammatory response may induce maturation of immature DC and promote migration, it is possible that a more severe inflammatory response impairs migration of mature DC out of skin.

Despite the finding that 1% of lymph node DC were derived from the injected population, migration was relatively inefficient, as only ~0.1% of injected cells could be accounted for in the draining lymph node at 36 h postinjection. Similar quantitative studies in the murine system also indicate that only 0.3% of injected DC can subsequently be detected in the draining lymph node (27). The reasons for this apparent inefficiency are not known. A large number of mature DC in our study could be accounted for in the dermis; however, the fate of immature DC that migrated completely from the skin but were not detected in the draining lymph node is uncertain. We are able to rule out the possibility that DC localized in substantial numbers to other nodes.
in the inguinal chain, as secondary nodes were collected and found to contain few, if any, labeled cells (data not shown). It is possible that injected cells trafficked via blood to distant lymphoid sites. Labeled cells were not detected in venous blood samples at 30 min and 36 h postinjection in several experiments (data not shown), although massive dilution by circulating leukocytes would make such cells very difficult to detect.

Our studies with membrane-labeled DC are limited by the fact that the dye does not represent a model Ag, and hence the trafficking results do not relate directly to DC-based immunotherapy. However, we showed, using a recombinant adenosivirus, that DC transduced in vitro with EGFP traffic to lymph nodes relatively efficiently and retain high level expression for at least 36 h. These data clearly support the application of adenaloviral vectors as a means of loading DC with Ag for vaccination protocols (35–37). By comparison, DC transfected in situ with EGFP using a gene gun were not detectable in draining lymph nodes by flow cytometry, although single EGFP-transfected cells could be detected in skin and lymph node by fluorescence microscopy. This would suggest a frequency of EGFP+ DC that migrated to lymph nodes following gene gun administration to be <0.05% of total lymph node DC, at least 20-fold lower than that of ex vivo transduced DC. Although the number of directly transfected DC that localize in lymph nodes is relatively small, as has been shown by others in the murine system (26), we and others have shown that this method clearly represents a potent means of inducing Ag-specific immune responses (17, 26). Our data indicate that it is possible to generate substantially larger numbers of gene-transfected DC in draining lymph nodes using ex vivo manipulation compared with gene gun administration. Whether this difference translates into a stronger immune response is yet to be determined.

Our results suggest that DC-based vaccine protocols can be substantially more flexible with respect to in vitro maturation than was previously thought. It may be preferable to administer immature DC immediately following Ag pulsing, especially when Ag is supplied as exogenous peptide that may have a limited half-life. Factors in the dermis will induce maturation of injected cells, leading to responsiveness to constitutive chemokines and migration into lymphatics and lymph nodes. In contrast, our finding that mature DC remain at the site of injection in such large numbers suggests that a majority of in vitro matured DC may not contribute effectively to the immune response. It will be important to determine in this model the relative immunogenicities of vaccines using immature and mature DC as well as cutaneous immunization via gene gun to establish the optimal DC-based vaccine system for human use.

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References


