

# Reducing cell number improves the homing of dendritic cells to lymph nodes upon intradermal vaccination

Erik HJG Aarntzen<sup>1,2,3</sup>, Mangala Srinivas<sup>1</sup>, Gerty Schreibelt<sup>1</sup>, Arend Heerschap<sup>4</sup>, Cornelius JA Punt<sup>5</sup>, Carl G Figdor<sup>1</sup>, Wim J Oyen<sup>3</sup>, and I Jolanda M de Vries<sup>1,\*</sup>

<sup>1</sup>Department of Tumor Immunology; Nijmegen Centre for Molecular Life Sciences; Radboud University Nijmegen Medical Centre; Nijmegen, The Netherlands;

<sup>2</sup>Department of Medical Oncology; Radboud University Nijmegen Medical Centre; Nijmegen, The Netherlands; <sup>3</sup>Department of Nuclear Medicine; Radboud University Nijmegen Medical Centre; Nijmegen, The Netherlands; <sup>4</sup>Department of Radiology; Radboud University Nijmegen Medical Centre; Nijmegen, The Netherlands;

<sup>5</sup>Department of Medical Oncology; Academic Medical Centre; Amsterdam, The Netherlands

**Keywords:** dendritic cell, imaging, migration, immunotherapy, <sup>19</sup>F MRI

Dendritic cell (DC)-based vaccines require the cells to relocate to lymph nodes (LNs). Unfortunately, however, DC migration rates are typically very poor. We investigated strategies to increase the migration efficacy of DC-based vaccines. Surprisingly, a reduction in DC number, but not the conditioning of the injection site, improved LN targeting.

Anticancer vaccines based on dendritic cells (DC) loaded *ex vivo* with tumor-associated antigens require these cells to relocate from the injection site to immunoreactive sites for triggering an effective immune response. The migration rates of DCs to lymph nodes (LNs), however, are generally low and highly dependent on the route of administration.<sup>1</sup> The intradermal injection of DCs is the strategy most frequently employed in clinical studies, but the number of cells that reach a single LN has never reproducibly exceeded 4% of the total amount of cells injected.<sup>1</sup> Several reasons have been suggested to account for the poor migration rate of mature DCs, including the lack of a proper inflammatory microenvironment that would promote the emigration of immune cells to afferent lymphatic vessels. In mice, the pretreatment of the skin with pro-inflammatory cytokines has been shown to provoke a 5–10-fold increase in the number of DCs that reach the draining LNs, resulting in a similar improvement in T-cell activation.<sup>2</sup> The frequency of DC delivery, the conditions of the vascular and lymphatic networks at site of inoculation and the local availability of oxygen and nutrients have also been

suggested to play an important role in this setting.<sup>3</sup> Thus, conditioning the injection site, and perhaps indirectly draining LNs, may significantly improve the clinical efficacy of DC-based immunotherapy.

We have recently investigated DC migration upon the intradermal delivery of radioactively (<sup>111</sup>In)-labeled DCs to metastatic melanoma patients participating in an ongoing clinical study.<sup>4</sup> Scintigraphic imaging demonstrated that the migration of DCs to LNs mainly occurs within the first 24 h after intradermal vaccination. The establishment of local inflammation by pre-treating the injection site with activated DCs, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) or the synthetic Toll-like receptor (TLR)7/8 agonist Imiquimod slightly enhanced the migration rate of injected DC. However, migration did not significantly increase in conditioned vs. unconditioned sites of the same patient, and the amount of cells reaching LNs did not exceed 4% of total injected cells. We have previously shown that a large part of injected DCs die at the inoculation site and are cleared by freshly recruited macrophages.<sup>4,5</sup> However, the co-injection of granulocyte macrophage

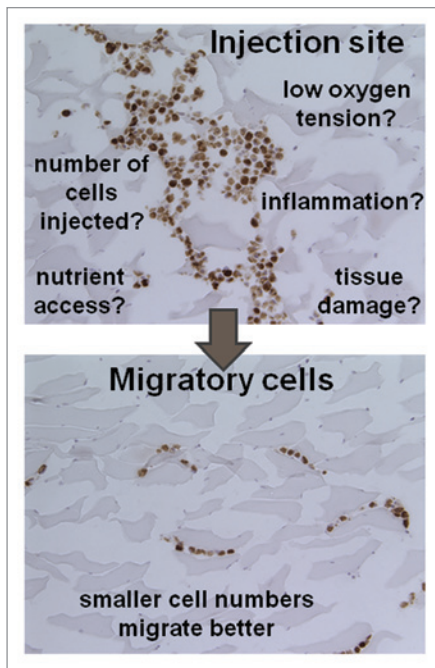
colony-stimulating factor (GM-CSF) to enhance DC survival also did not significantly improve DC migration rates. Of note, the induration of injection sites was markedly larger upon the co-injection of DCs and GM-CSF than after the administration of DCs alone, suggesting that GM-CSF stimulates the random migration of DCs into the surrounding dermis.

Patient availability and ethical considerations hamper large *in vivo* migration studies in humans. *In vitro* models overcome these issues and provide a way to optimize multiple parameters that may influence migration rates. Some drawbacks of commonly exploited cell migration *in vitro* assays, which are often microscopy- or microtiter plate-based, limit their translational relevance. These techniques typically work only with small numbers of cells and/or non-opaque samples. Furthermore, most of these methods assess cell migration in a 2-dimensional setting, whereas *in vivo* migration entails 3D motility. To test the hypothesis that local cell density would constitute the key factor limiting DC migration upon intradermal delivery, we modified an *in vitro* assay that closely reflects *in vivo* vaccination conditions

\*Correspondence to: I Jolanda M de Vries; Email: j.devries@ncmls.ru.nl

Submitted: 04/10/13; Accepted: 04/11/13

Citation: Aarntzen EHJG, Srinivas M, Schreibelt G, Heerschap A, Punt CJA, Figdor CG, et al. Reducing cell number improves the homing of dendritic cells to lymph nodes upon intradermal vaccination. *Oncoimmunology* 2013; 2:e24661; <http://dx.doi.org/10.4161/onci.24661>



**Figure 1.** Factors influencing the migration of dendritic cells from the injection site to lymph nodes. Several factors can affect dendritic cell (DC) migration from the injection site to draining lymph nodes (LNs). These histological sections show a tissue injected *ex vivo* with DCs stained with a marker of hypoxia (brown, upper panel) and groups of migratory cells (lower panel). The inoculation site is particularly prone to problems such as those listed in the figure. Our findings indicate that the injection of low numbers of DCs can alleviate these issues and favor DC migration to LNs.

to measure human DC migration in a standardized manner in tissue samples.<sup>6</sup> Thanks to this model, we were able to quantify the CCL21-directed migration of <sup>19</sup>F-labeled DC-based vaccines over a prolonged period using <sup>19</sup>F magnetic resonance imaging (MRI), which allows for

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the direct quantification of cell numbers from imaging data.<sup>7</sup> Of note, the <sup>19</sup>F particles used to label DCs are not toxic and do not affect their migration.<sup>8</sup>

Using this assay, we demonstrated that increasing the cell density indeed suppresses the 3D migration of DCs toward a source of CCL21 *in vitro*.<sup>4</sup> We obtained similar results in patients receiving a DC-based vaccine, a setting in which the average percentage of migratory DCs increased significantly when the number of DCs per inoculation was reduced by using multiple injection sites. When we compared the migration data that we obtained *in vitro* using our <sup>19</sup>F MRI-based assay with the clinical data obtained by means of scintigraphy on <sup>111</sup>In-labeled DCs, we found that a comparable percentage of migratory DCs *in vivo* and *in vitro*, when low number of cells were used. However, due to the sensitivity limits of clinical scintigraphy, very small numbers of migratory DCs cannot be detected with current clinical imaging techniques.

In the past decades, various parameters of DC-based vaccination have been optimized. At this point, the paradigm is shifting from small proof-of-principle studies to large, randomized, and controlled clinical trials. Accordingly, the feasibility and efficacy of cellular immunotherapy on a large scale is now the true focus of attention. Although the intradermal route of administration is generally the easiest approach, and therefore preferred in most clinical trials, limited numbers of DCs reach draining LNs in this setting. Of note, the optimal amount of DCs per LN for the induction of adequate antitumor immune responses in humans has not yet

been established. Some studies report a dose-dependent relationship between the amounts of intranodal DCs and immune responses in humans, while others suggest that small numbers of antigen-loaded DCs are sufficient to elicit robust immune responses.<sup>5,9,10</sup> Given the elevated immunostimulatory potential of DCs *in vitro*, this latter hypothesis appears as the most plausible. We have previously shown that the intradermal—as compared with the intranodal—administration of DCs induce superior tumor-specific immune responses in melanoma patients.<sup>10</sup> Hypothetically, unfavorable conditions at the site of delivery may allow only the fittest DCs, exhibiting high immunostimulatory activity, to migrate to LNs and induce immune responses (Fig. 1). Thus, we suggest that multiple intradermal injections with small amounts of DCs, *de facto* targeting multiple lymph nodes, would increase the clinical efficacy of DC-based immunotherapy.

## Acknowledgements

This work was supported by grants KUN 2008/035 from the Dutch Cancer Society, Netherlands Organization for Scientific Research (NWO), grants 920-03-250 and NWO-Veni 700.10.409, NWO-Vidi-917.76.363, AGIKO-92003250, The Radboud University Nijmegen Medical Centre AGIKO-2008-2-4, the ATK foundation, ENCITE, NWO Spinoza award, and a European Research Council (ERC) grant ERC-2010-AdG-269019-PATHFINDER and KWF2009-4402.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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