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Migration of Dendritic Cells from Murine Skeletal Muscle

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Abstract

To better understand the role of dendritic cells (DCs) in skeletal muscle, we investigated the migration of DCs from murine skeletal muscle and compared that to previously studied footpad (FP) DC trafficking. We adoptively transferred carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled mature DCs to syngeneic mice and followed them in various lymphatic tissues at different time points. Injection of DCs into the tibialis anterior muscle resulted in the peak number of CFSE⁺ DCs recovered in spleen at 12h, not at 24h, when the largest number of these cells appeared in the draining lymph nodes. Interestingly, this result for adoptive transfer of DCs to skeletal muscle differs with what is previously reported for adoptive transfer to the FP, a result that we also confirmed in parallel studies. These findings could have a significant impact on 1) understanding muscle diseases with immunological complications such as muscular dystrophies and 2) the immunologic effects of treatments for muscle diseases.

Keywords

muscle; skeletal; dendritic cells; cell trafficking

INTRODUCTION

Among professional antigen presenting cells (APCs), dendritic cells (DCs) are the most effective in antigen presentation and are likely the principal mediators of the deleterious immune reaction observed in inflammatory diseases including inflammatory myopathies. The capability of DCs to initiate a primary immune response is provided by expression of lymphocyte costimulatory molecules, migration to lymphoid organs, and secretion of biologically active molecules such as leukocyte-recruiting chemokines. Therefore, DCs may be excellent targets for efforts to modulate the immunological events related to a disease or a treatment in any tissue, including skeletal muscle.

An adaptive immune response is mediated by APCs through activation of T cells resulting in T cell and B cell-mediated immunity.^{1,2} DCs are the most potent APCs in the mammalian immune system because 1) they are the only APC that can prime naïve T cells and carry multiple types of information, including antigen specificities as well as cues about the nature of the attacking pathogen and the extent of host damage,^{1,3} and 2) they are highly mobile facilitating rapid communication between peripheral tissues and secondary lymphoid

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other remote organs.^{4,5} It is crucial to understand the temporal DC behavior in various tissues in order to successfully treat diseases and to modulate the immune system to promote or inhibit an immune response.

Inflammatory cells infiltrating skeletal muscle form a major component of inflammatory myopathies and also contribute to the pathogenesis of muscular dystrophies, such as Duchenne muscular dystrophy (DMD). Furthermore, treatment of dystrophic muscle with gene transfer designed to ameliorate single gene disorders affecting muscle in mouse models (eg. the *mdx* mouse modeling DMD) may increase muscle infiltration by immune cells.^{6–12} Immunity is a major hurdle preventing successful gene therapy for DMD and other muscle diseases.

In order to successfully modulate DCs during treatments of skeletal muscle, it is of fundamental importance to study the migration patterns of muscle DCs. Interference with DC trafficking from tissues to their target organs often leads to marked defects in the initiation of immune responses.^{13–18} Therefore, in this study we explored the migration of labeled bone marrow (BM)-derived DCs (BMDCs) that were adoptively transferred to syngeneic murine skeletal muscle using previously published procedures.^{4,19} We compared our findings to DC trafficking from the footpad (FP), which has been studied previously, to better understand potential differences in DC migration patterns.

MATERIALS AND METHODS

Mice

Male C57BL/10, C57BL/6 (CD45.2/Ly5.2) and congenic CD45.1/Ly5.1 (B6.SJL-*Ptprca^uPep3^b/BoyJ*) mice aged 6 weeks were purchased from The Jackson Laboratory. Animal use for these studies was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Generation and purification of BMDCs

BMDCs were propagated as described with some modifications.¹² Briefly, BM cells were harvested from femurs and tibias of C57BL/10 or C57BL/6 mice. Erythrocytes were lysed with 0.83% w/v NH₄Cl buffer, and leukocytes depleted with a monoclonal antibody cocktail consisting of rat IgG anti-B220, anti-CD3, anti-ter-119, anti-Ly-6G and Ly-6C (all from BD Pharmingen; Franklin Lakes, NJ) plus rabbit complement (American Chemical and Scientific Corporation, Westbury, NY). The cells were resuspended at 1×10^6 cells/ml completed medium (RPMI-1640 (Gibco, Co., Grand Island, NY) supplemented with 10% v/v FBS, 50 μ M β -mercaptoethanol (2-ME), 2mM glutamine, 0.1 mM non-essential amino acids, 100 μ g/ml streptomycin and 100 IU/ml penicillin) with 1000 U/ml recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) (BD Pharmingen) and 1000 U/ml recombinant murine interleukin-4 (IL-4) (BD Pharmingen). Cells were seeded in 1 ml aliquots in 24-well culture plates. At 3d, non-adherent cells were removed carefully by gentle tapping of the plates, and half of the medium was replaced with fresh complete media containing GM-CSF and IL-4. Cells were fed at 5d. Then at 7d, loosely adherent cells were harvested by gentle pipetting, and CD11c⁺ cells were sorted after labeling with immunobead-conjugated anti-mouse CD11c mAb (Miltenyi Biotec, Auburn, CA) followed by passage through a positive selection paramagnetic column (Miltenyi Biotec).

Spleen and lymph node (LN) DC isolation

To analyze the number of migrating DCs, popliteal and inguinal LNs and spleens were minced into small fragments and treated with 1 mg/ml collagenase-D (Roche Molecular Biochemicals, Indianapolis, IN) at 37°C for 45 min. Cells were passed through a mesh,

depleted of erythrocytes by adding 0.83% w/v NH_4Cl buffer and then resuspended in ~8 ml RPMI/~ 8×10^7 cells. Eight ml of cell suspension was gently layered over 2 ml 16.5% Histodenz (Sigma-Aldrich, St. Louis, MO), and lymphocytes were purified by centrifugation on a Histodenz gradient.

Flow cytometry

DCs were characterized by flow cytometric analysis of cell surface antigen expression. Briefly, cells were stained with directly-conjugated mAbs that recognized CD80, CD86, CD11c or appropriate isotype-matched Igs (all from BD Pharmingen). After staining, the cells were fixed with 2% (w/v) paraformaldehyde and analyzed using a Coulter EPICS XL.MCL (Beckman Coulter) flow cytometer and EXPO 32 software (Applied Cytometry Systems, Sheffield, UK).

In vivo migration

Immunobead-sorted DCs were labeled for 20 min at 37°C with 5 μM 5- and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR). Following incubation, labeling was stopped by the addition of RPMI/10% FBS and cells were washed 3 times with RPMI at room temperature. To reduce the amount of unbound CFSE in cell suspensions, the cells were incubated at 37°C for 5 min after the second wash and prior to the third wash. The CFSE-labeled DCs were injected into both hind FP or both TA muscles of syngeneic mice. FP-recipient and TA-recipient mice received $2\text{--}5 \times 10^6$ cells in 50 μl PBS, and 5×10^6 cells in 40 μl PBS, respectively. At different time points between 2 and 48h, the popliteal and inguinal LNs, spleen, and BM were removed. DCs were isolated and analyzed by flow cytometry as described above. Adoptively transferred DCs were identified by the CFSE marker.

In vivo migration pathways of DCs following TA muscle injection or FP injection was also studied by virtue of their expression of distinct congenic marker CD45.1 or CD45.2. BMDCs derived from CD45.2 donor mice were transferred into congenic CD45.1 recipient mice. Subsequently, donor DCs that had homed to recipient spleen and LNs were identified by coexpression of CD45.2 and CD11c.

RESULTS

Characterization of BMDCs

DCs were isolated from BM of C57BL/10 or C57BL/6 (CD45.2) donor mice, cultured with IL-4 and GM-CSF for 7d, and purified by passage over anti-CD11c antibody-coated magnetic beads. After purification, approximately 85% of cells expressed both CD11c and CD86, which indicated that they were mature DCs (Figure 1a). After labeling of C57BL/10 DCs with CFSE, there was no change in the phenotypic expression of CD11c and CD86 (Figure 1b). Other studies also demonstrate that a high proportion of DCs have a mature phenotype after *ex vivo* purification.^{20,21}

In vivo migration pathways of CFSE-labeled DCs after FP injection

Numerous studies have investigated DC migration after FP injection, but their trafficking at early time points has not been documented. Furthermore, our interest was to compare DC migration from muscle to DC migration from the FP. We performed parallel experiments with either adoptive transfer of CFSE-labeled C57BL/10 DCs to C57BL/10 mice or C57BL/6 (CD45.2) DCs to congenic C57BL/6 (CD45.1) mice. Similar results were obtained in the parallel experiments. In Figure 2 we show data from a representative experiment with CFSE-labeled C57BL/10 DCs.

We analyzed DC migration at 24 and 48 hours post-cell injection (referred to as 1D and 2D, respectively). Following FP injection of CFSE-labeled DCs, the transferred cells were found in popliteal and inguinal LNs and spleen. The number of these cells increased in a time-dependent manner in popliteal LNs (Figure 2a). At 2 days after adoptive transfer of the cells, most recovered CFSE⁺ DCs were found in popliteal LNs, with fewer CFSE⁺ DCs found in inguinal LNs (Figure 2b). There were very few DCs recovered from spleen at 1 or 2 days post-DC transfer (Figure 2b). This study confirmed the results of others showing that CFSE-labeled DCs adoptively transferred to FP migrated to draining LNs, then traveled to the spleen from the blood stream through the efferent lymphatics.^{5,22}

In vivo migration pathways of CFSE-labeled DCs after skeletal muscle injection

We examined the movement of C57BL/10 BM-derived CFSE-labeled DCs that had been adoptively transferred to TA muscles of syngeneic mice at the same time-points as the experiments with adoptive transfer of DCs to the FP, ie. 2, 12, 24, and 48 hours (referred to as 2h, 12h, 1D, and 2D, respectively) post-cell transfer. Quantification of FACS data showed that as early as 2h post-cell transfer CFSE⁺ DCs were recovered from inguinal and popliteal LNs (Figure 3c). By 12h post-adoptive transfer of DCs, a larger number of DCs migrated to inguinal and popliteal LNs. Labeled DCs migrating from TA increased considerably in inguinal and popliteal LNs at 1 day (Figure 3c). We recovered the largest number of CFSE⁺ DCs from popliteal LNs, a smaller number of these cells from inguinal LNs, and very few from BM and spleen at this time point (Figure 3a and c). At 2 days, these cells were reduced in both inguinal and popliteal LNs (Figure 3b and Figure 3c), and were undetectable thereafter (data not shown).

The migration pattern to the spleen from the TA differed from the migration pattern to the spleen from the FP. At 12h we observed the peak level of CFSE⁺ DCs migrating to the spleen from the TA (Fig 3d). Over the next 36h the number of CFSE⁺ DCs in the spleen decreased. After 2 days, no CFSE⁺ DCs were found in the spleen (data not shown).

DISCUSSION

To provide a reference point with which to compare DC mobilization from skeletal muscle, we confirmed prior published studies showing that CFSE-labeled DCs adoptively transferred subcutaneously to the FP migrated to popliteal LNs and subsequently reached inguinal LNs. The majority of these cells stayed in draining LNs, while a very small fraction migrated from draining LNs to spleen through the thoracic duct.^{22,23}

Previously published studies demonstrate that DCs injected subcutaneously, intraperitoneally, or directly into the central nervous system preferentially home to the draining LNs,^{5,13,22,24–28} whereas DCs injected intravenously accumulate primarily in the spleen.^{5,28} Other studies suggest that antigen stimulation leads to migration of resident DCs in airway and small intestine to draining LNs primarily.²⁹ However, in some studies small numbers of labeled DCs were found in remote organs such as spleen, BM and thymus, indicating that DCs leave the tissues and may reach the bloodstream by way of draining LNs and efferent lymphatics.^{4,5}

Our current studies of DC migration from skeletal muscle showed significant differences from DC migration originating in the FP. We observed that adoptively transferred CFSE-labeled DCs moved to the spleen soon after adoptive transfer to skeletal muscle. The peak number of CFSE⁺DCs recovered in spleen occurred at 12h, not 24h after injection when the largest number of these cells appeared in the popliteal LNs. This finding suggests that a sub-fraction of the transferred DCs in skeletal muscle traveled through the bloodstream directly to spleen. Although there is ample experimental evidence that draining LNs are the terminal

targets for most DCs that leave peripheral tissues,^{13,22,24,26–28,30} recent investigations suggest that tissue-resident DCs can return to the blood and carry antigen to organs other than LNs.^{5,19,31} The simplest interpretation of our results is that muscle resident DCs reach the spleen by dissemination through the bloodstream, perhaps gaining access through the well-developed capillary network of skeletal muscle, without going through lymphatics. Another possibility is that DCs migrating from skeletal muscle could take on specific homing factors that confer tropism to the spleen. Previously published studies report that circulating DCs home to BM, facilitated by up-regulation on DCs of P-selectin, E-selectin and integrin $\alpha_4\beta_1$, which is the principal ligand for vascular cell adhesion molecule 1.⁵ The existence of a direct route of antigen transport to the spleen could have interesting implications for the immune mechanisms of many inflammatory, autoimmune muscle disorders and genetic treatment of muscle diseases such as DMD.

In our previous studies, we suppressed immunity and enhanced the effectiveness of gene transfer in dystrophic muscle by co-administering gene vectors expressing cytokines CTLA4Ig and CD40Ig that block costimulation of T and B cells, respectively.^{32,33} Based on this previous data, it is likely that interference with antigen presentation by DCs to T cells will beneficially suppress undesirable immunity as well.

In conclusion, our study of DC activation and trafficking from skeletal muscle yielded important differences in the pattern of DC trafficking from skeletal muscle compared to other tissues. The differences in DC migration path that we observed from skeletal muscle may have important implications for the immune barriers to viral vector-mediated gene transfer to skeletal muscle. Further studies could be performed of the effect of antigen stimulation on DC activation and their migration profiles from skeletal muscle. Furthermore, our studies suggest that muscle DCs may be an important target to modulate immunity and thus to facilitate successful gene transfer. Because efficient gene transfer for the treatment of muscular dystrophy is an ultimate goal and because other studies have suggested that DCs act coordinately to mediate the acute and chronic inflammatory aspects of dystrophic changes in DMD muscle,^{9,34} then it will also be important to study DC trafficking from dystrophic muscle.

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Abbreviations

CFSE	carboxyfluorescein diacetate succinimidyl ester
DC	dendritic cell
LN	lymph node
APC	antigen presenting cell
FP	Footpad

References

1. Mosmann TR, Livingstone AM. Dendritic cells: the immune information management experts. *Nat Immunol* 2004;5:564–566. [PubMed: 15164014]

2. Wells DJ, Ferrer A, Wells KE. Immunological hurdles in the path to gene therapy for Duchenne muscular dystrophy. *Expert Rev Mol Med* 2002;4:1–23. [PubMed: 14585159]
3. Wiendl H, Lautwein A, Mitsdorffer M, et al. Antigen processing and presentation in human muscle: cathepsin S is critical for MHC class II expression and upregulated in inflammatory myopathies. *J Neuroimmunol* 2003;138:132–143. [PubMed: 12742663]
4. Bonasio R, von Andrian UH. Generation, migration and function of circulating dendritic cells. *Curr Opin Immunol* 2006;18:503–511. [PubMed: 16777395]
5. Cavanagh LL, Bonasio R, Mazo IB, et al. Activation of bone marrow-resident memory T cells by circulating, antigen-bearing dendritic cells. *Nat Immunol* 2005;6:1029–1037. [PubMed: 16155571]
6. Acsadi G, Lochmuller H, Jani A, et al. Dystrophin expression in muscles of mdx mice after adenovirus-mediated in vivo gene transfer. *Hum Gene Ther* 1996;7:129–140. [PubMed: 8788164]
7. Floyd SS Jr, Clemens PR, Ontell MR, et al. Ex vivo gene transfer using adenovirus-mediated full-length dystrophin delivery to dystrophic muscles. *Gene Ther* 1998;5:19–30. [PubMed: 9536261]
8. Gilchrist SC, Ontell MP, Kochanek S, et al. Immune response to full-length dystrophin delivered to Dmd muscle by a high-capacity adenoviral vector. *Mol Ther* 2002;6:359–368. [PubMed: 12231172]
9. Hartigan-O'Connor D, Kirk CJ, Crawford R, et al. Immune evasion by muscle-specific gene expression in dystrophic muscle. *Mol Ther* 2001;4:525–533. [PubMed: 11735336]
10. Howell JM, Lochmuller H, O'Hara A, et al. High-level dystrophin expression after adenovirus-mediated dystrophin minigene transfer to skeletal muscle of dystrophic dogs: prolongation of expression with immunosuppression. *Hum Gene Ther* 1998;9:629–634. [PubMed: 9551611]
11. Lochmuller H, Petrof BJ, Pari G, et al. Transient immunosuppression by FK506 permits a sustained high-level dystrophin expression after adenovirus-mediated dystrophin minigene transfer to skeletal muscles of adult dystrophic (mdx) mice. *Gene Ther* 1996;3 :706–716. [PubMed: 8854096]
12. Yang Y, Haecker SE, Su Q, et al. Immunology of gene therapy with adenoviral vectors in mouse skeletal muscle. *Hum Mol Genet* 1996;5:1703–1712. [PubMed: 8922997]
13. Angeli V, Staumont D, Charbonnier AS, et al. Activation of the D prostanoid receptor 1 regulates immune and skin allergic responses. *J Immunol* 2004;172:3822–3829. [PubMed: 15004188]
14. Berghofer B, Haley G, Frommer T, et al. Natural and synthetic TLR7 ligands inhibit CpG-A- and CpG-C-oligodeoxynucleotide-induced IFN- α production. *J Immunol* 2007;178:4072–4079. [PubMed: 17371961]
15. Chen X, Yang L, Howard OM, et al. Dendritic cells as a pharmacological target of traditional Chinese medicine. *Cell Mol Immunol* 2006;3:401–410. [PubMed: 17257493]
16. Del PA, Shao WH, Mitola S, et al. Regulation of dendritic cell migration and adaptive immune response by leukotriene B4 receptors: a role for LTB4 in up-regulation of CCR7 expression and function. *Blood* 2007;109:626–631. [PubMed: 16985179]
17. Hackstein H, Thomson AW. Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. *Nat Rev Immunol* 2004;4:24–34. [PubMed: 14704765]
18. Mahnke K, Qian Y, Knop J, et al. Dendritic cells, engineered to secrete a T-cell receptor mimic peptide, induce antigen-specific immunosuppression in vivo. *Nat Biotechnol* 2003;21 :903–908. [PubMed: 12833070]
19. Randolph GJ, Angeli V, Swartz MA. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat Rev Immunol* 2005;5:617–628. [PubMed: 16056255]
20. Croizet K, Rabilloud R, Kostrouch Z, et al. Culture of dendritic cells from a nonlymphoid organ, the thyroid gland: evidence for TNF α -dependent phenotypic changes of thyroid-derived dendritic cells. *Lab Invest* 2000;80:1215–1225. [PubMed: 10950112]
21. Fearnley DB, McLellan AD, Mannering SI, et al. Isolation of human blood dendritic cells using the CMRF-44 monoclonal antibody: implications for studies on antigen-presenting cell function and immunotherapy. *Blood* 1997;89:3708–3716. [PubMed: 9160676]
22. Eggert AA, Schreurs MW, Boerman OC, et al. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. *Cancer Res* 1999;59 :3340–3345. [PubMed: 10416590]
23. Eggert AA, van d V, Torensma R, et al. Analysis of dendritic cell trafficking using EGFP-transgenic mice. *Immunol Lett* 2003;89:17–24. [PubMed: 12946860]

24. Barratt-Boyes SM, Watkins SC, Finn OJ. In vivo migration of dendritic cells differentiated in vitro: a chimpanzee model. *J Immunol* 1997;158:4543–4547. [PubMed: 9144465]
25. Baumjohann D, Lutz MB. Non-invasive imaging of dendritic cell migration in vivo. *Immunobiology* 2006;211:587–597. [PubMed: 16920497]
26. Baumjohann D, Hess A, Budinsky L, et al. In vivo magnetic resonance imaging of dendritic cell migration into the draining lymph nodes of mice. *Eur J Immunol* 2006;36 :2544–2555. [PubMed: 16909432]
27. Karman J, Ling C, Sandor M, et al. Initiation of immune responses in brain is promoted by local dendritic cells. *J Immunol* 2004;173:2353–2361. [PubMed: 15294948]
28. Lappin MB, Weiss JM, Delattre V, et al. Analysis of mouse dendritic cell migration in vivo upon subcutaneous and intravenous injection. *Immunology* 1999;98:181–188. [PubMed: 10540216]
29. Novak N, Allam JP, Betten H, et al. The role of antigen presenting cells at distinct anatomic sites: they accelerate and they slow down allergies. *Allergy* 2004;59:5–14. [PubMed: 14674927]
30. MartIn-Fontecha A, Sebastiani S, Hopken UE, et al. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. *J Exp Med* 2003;198:615–621. [PubMed: 12925677]
31. Legge KL, Braciale TJ. Accelerated migration of respiratory dendritic cells to the regional lymph nodes is limited to the early phase of pulmonary infection. *Immunity* 2003;18:265–277. [PubMed: 12594953]
32. Jiang Z, Schiedner G, Gilchrist SC, et al. CTLA4Ig delivered by high-capacity adenoviral vector induces stable expression of dystrophin in mdx mouse muscle. *Gene Ther* 2004;11 :1453–1461. [PubMed: 15269713]
33. Jiang ZL, Reay D, Kreppel F, et al. Local high-capacity adenovirus-mediated mCTLA4Ig and mCD40Ig expression prolongs recombinant gene expression in skeletal muscle. *Mol Ther* 2001;3:892–900. [PubMed: 11407903]
34. Chen HH, Mack LM, Choi SY, et al. DNA from both high-capacity and first-generation adenoviral vectors remains intact in skeletal muscle. *Hum Gene Ther* 1999;10:365–373. [PubMed: 10048389]

Figure 1. Characterization of bone marrow-derived DCs

Bone marrow cells from C57BL/10 mice were differentiated into mature dendritic cells (DCs) *in vitro*. (a) After purification, DCs were stained with FITC-labeled anti-CD11c and PE-labeled CD86 antibodies as indicated. (b) After 5-and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) staining, DCs were stained with PE-CD11c or PE-labeled CD86 antibodies. The data shown are representative of >15 separate experiments.

Figure 2. *In vivo* migration pathways of CFSE-DCs after FP injection

Purified, 5- and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled mature dendritic cells (DCs) were injected subcutaneously into both hind limb foot pads (FP) of syngeneic C57BL/10 mice. (a) CFSE-labeled DCs recovered in popliteal lymph nodes (LNs) at each time point (h, hour; D, day). (b) Results of recovered DCs quantified by FACS show the organ and time distribution of adoptively transferred DCs. The absolute number of DCs that homed to spleen, inguinal LN (ing LN) and popliteal LN (pop LN) was calculated by multiplication of the frequency of $CD11c^+$ transferred cells by the total number of resident leukocytes. Data are presented as number of recovered DCs per 5×10^5 injected cells. The data shown represent simultaneous adoptive transfer to a group of mice and collection of one mouse at each time-point. The temporal and target tissue destination patterns shown are representative of 6 independent experiments.

Figure 3. *In vivo* migration pathways of CFSE-DCs after TA muscle injection

Purified, 5- and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled dendritic cells (DCs) were injected into both tibialis anterior (TA) muscles of syngeneic C57BL/10

mice. After 1 day (a) or 2 day (b), popliteal lymph node (pop LN), inguinal LN (ing LN), spleen and bone marrow (BM) were collected to determine the number of CFSE⁺ DCs in each of these organs. Quantification of FACS results show the distribution of CFSE-labeled DCs by the anatomical location of LNs (c) and spleen (d) and time (h, hour; D, day) after adoptive transfer to TA muscle. Data are presented as number of recovered DCs per 5×10^5 injected cells. The data shown represent simultaneous adoptive transfer to a group of mice and collection of one mouse at each time-point. The temporal and target tissue destination patterns shown are representative of 6 independent experiments.