Original Article

마우스에서 Tc-99m HMPAO 표지 미성숙 및 성숙 수지상세포의 이동에 관한 연구

전남대학교 의과대학 핵의학교실[1], 전남대학교 의과대학 내과학교실[2], 전남대학교 의과대학 외과학교실[3], 전남대학교병원 임상연구소[4]
이명호[1] · 이제중[2] · 민정준[1] · 허영준[1] · 송호천[1] · 박영규[3] · 박안나[4] · 범희승[1]

Migration of ^{99m}Tc-Hexamethylpropylene Amine Oxime (HMPAO) Labeled Immature and Mature Dendritic Cells in the Mouse

Ming-Hao Li, M.D.[1], Je-Jung Lee, M.D.[2], Jung-Joon Min, M.D.[1], Young-Jun Heo, M.D.[1], Ho-Chun Song, M.D.[1], Young-Kyu Park, M.D.[3], Anna Park, M.D.[4], Hee-Seung Bom, M.D.[1],
Department of Nuclear Medicine, Chonnam National University Medical School, Gwangju, Korea[1]
Department of Medicine, Chonnam National University Medical School, Gwangju, Korea[2]
Department of Surgery, Chonnam National University Medical School, Gwangju, Korea[3]
Research Institute for Clinical Medicine, Chonnam National University Hospital, Gwangju, Korea[4]

Purpose: The purpose of this study is to evaluate migration of technetium-99m hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) labeled immature and mature dendritic cells (DC) in the mouse. **Methods:** DC were collected from bone marrow (BM) of tibiae and femurs of mice. Immature and mature DC from BM cells were radiolabeled with ^{99m}Tc-HMPAO. To evaluate the functional and phenotypic changes of DC from radiolabeling, the allogeneic mixed lymphocyte reaction (MLR) and fluorescence-activated cell sorting (FACS) analysis were performed before and after labeling with ^{99m}Tc-HMPAO. Migration of intravenously injected DC (iv-DC) was assessed by serial gamma camera images of mice with or without subcutaneous tumor. Percent injected dose per gram (%ID/g) was calculated in lungs, liver, spleen, kidneys, and tumor through dissection of each mice after 24 hours of injection. **Results:** Labeling efficiency of immature and mature DC were 60.4±5.4% and 61.8±6.7%, respectively. Iv-DC initially appeared in the lungs, then redistributed mainly to liver and spleen. Migration of mature DC to spleen was significantly higher than that of immature DC (38.3±4.0 % vs. 32.2±4.1 % in control group, 40.4±4.1% vs. 35.9 ±3.8 % in tumor group; $p<0.05$). Migration to tumor was also significantly higher in mature DC than in immature DC (2.4±0.3% vs 1.7±0.2%; $p=0.034$). **Conclusion:** Assessment of migration pattern of DC in mice was possible using ^{99m}Tc-HMPAO labeled immature and mature DC. Migration of mature DC to spleen and tumor was higher than that of immature DC when they were i.v. injected.(Korean J Nucl Med 39(1):26-33, 2005)

Key Words: Radionuclide imaging, Dendritic cell, Migration, ^{99m}Tc-HMPAO, Mouse

Introduction

Dendritic cells (DC) represent an heterogeneous family of cells which function as sentinels of the immune system. They traffic from the blood to the tissues where, while immature, they capture antigens. On activation by antigenic challenge and/or inflammation, they leave the tissues and move to the draining lymphoid organs where, converted into mature DC, they prime naive T cells.[1] Thus, correct functioning as antigen-presenting cells (APCs) includes appropriate trafficking in tissues and localization to lymph nodes (LN) and spleen.[2-4] Currently, clinical trials of cancer immunotherapy using autologous peripheral blood DC are underway.[4-6] However, the best route of DC administration for ensuring migration to T cell-rich sites is uncertain.[7] To evaluate the migration route of administered DC, radiolabeling of DC is

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• **Address for reprints**: Hee-Seung Bom, M.D., Ph.D., Department of Nuclear Medicine, Chonnam National University Hwasun Hospital, #160 Ilsim-ri, Hwasun, Chonnam 519-809, Korea
Tel: 82-61-379-7270, Fax: 82-61-379-7280
E-mail: hsbom@jnu.ac.kr
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mandatory.

Compared to other radiolabeled agents, ^{99m}Tc labeled agents have favourable physical characteristics and several advantages for gamma camera imaging. They permit rapid imaging, good image resolution and readily available in routine clinical setting for all nuclear medicine laboratories because of a product of generator system. Hexamethylpropyleneamine oxime (HMPAO) is a small, neutral, and lipophilic complex that binds ^{99m}Tc.[8] The lipophilicity of HMPAO allows it to cross the cell membrane, where it remains fixed. After crossing the cell membrane, the primary ^{99m}Tc-HMPAO complex changes into a hydrophilic secondary complex that is trapped within the cell. The radioactivity is bound to intracellular organelles, primarily the mitochondria and nucleus.[9]

The purpose of this study is to evaluate migration of ^{99m}Tc-HMPAO labeled immature and mature dendritic cells (DC) in the mouse. For that, both immature and mature DC derived from bone marrow of mice were radiolabeled using ^{99m}Tc-HMPAO. They were administered into mice *intravenously*. Migration of radiolabeled DC was monitored by gamma camera imaging. Biodistribution of radiolabeled DC in mice was observed by gamma counting of tissues. Whether or not DC exhibit different migration patterns in mouse tumor model was also observed.

Materials and Methods

1. Generation of immature DC from bone marrow (BM) of mice and induction of mature DC

Six week old male Balb/c mice weighing 20-30 gram (Semtako Bio Korea, Osan, Korea) were used, acclimated for 1 week and fed a diet of animal chow and water *ad libitum*. After removing all muscle tissues from the femurs and tibiae of mice, the bones were placed in a 60 mm dish with 70% alcohol for 1 minute, washed twice with phosphate buffered solution (PBS), and transferred into afresh dish with RPMI 1640. Both ends of the bones were cut with scissors in the dish, and then was flushed out using 2 ml of RPMI 1640. The tissue was suspended, passed through nylon mesh to remove small pieces of bone and debris, and red cells were lysed with ammonium chloride.

After washing, lymphocytes (T-cells) were collected for mixed lymphocyte reaction (MLR). 7-10$\times10^5$ cells were placed in 12-well plates (NUNC^{TM}, Denmark) in RPMI 1640 (GIBCO-BRL; Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (FBS), and 1% penicillin-streptomycin.

To generate immature DC, the bone marrow cells were cultured with RPMI 1640 containing 10% FBS in the presence of 50 ng/ml mouse granulocyte-macrophage colony-stimulating factor (rmGM-CSF), 50 ng/ml mouse interleukin-4 (rmIL-4), and freshly prepared cytokines added every other day. Maturation of DC was induced by adding 50 ng/ml tissue necrosis factor (TNF-α) on day 6. DC were harvested as immature (day 6) or mature (day 9) cells for phenotypic, or functional evaluations.[10]

2. ^{99m}Tc-HMPAO labeling of DC

DC were rapidly resuspended at a concentration of 10^7 DC/100 μl of RPMI 1640 containing 10% FBS. DC labeling was obtained by adding 185 MBq of ^{99m}Tc-HMPAO (Amersham Health^{TM}, England) to 10^7 DC. DC were incubated for 30 min with the tracer at room temperature, and than washed twice with the medium. Labeling efficiency (LE) was determined by counting the activity linked to the cells and in the supernatant. LE was the amount of radioactivity in the cells divided by total radioactivity.

3. Allogeneic mixed lymphocyte reaction (MLR)[11]

The allogeneic MLR was performed on the DC before and after labeling with ^{99m}Tc-HMPAO to determine whether the function of the DC was altered by radiolabeling. Mature DC was cultured in RPMI 1640 supplemented with 10% FBS on 96-well U-bottomed tissue culture plates. Irradiated (30 Gy) DC preparations in grade doses of 800-50,000 cells/well as well as T lymphocytes in a fixed dose of 5×10^4 cells/well in a total volume of 200 μl were added into the plate. Cell proliferation after 96 hours was quantified by adding 37 kBq (1 μCi) of ^{3}H-methyl thymidine (NEN-Dupot, USA) to each well. After 16 hours, the cells were harvested onto filters, and radioactivity was measured by a liquid

scintillation counter (LS 6500 Scintillation System, USA) and the results were presented as the mean counts per minute (cpm) for triplicate culture.

4. Flow cytometry

Fluorescence-activated cell sorting (FACS) analysis was performed before and after ^{99m}Tc-HMPAO labeling. FACS analysis was performed using the mouse monoclonal antibodies CD11c, CD80, CD86, and anti-mouse I-A^b monoclonal antibody (BD Biosciences). Isotype controls were mouse IgG_{2a}, k; IgG1, ; IgG2, k; and IgG_{2a}, k. The samples were acquired on a FACS Caliber instrument (Becton Dickinson, USA) and analyzed with CellQuest software (Becton Dickinson, USA).

5. Generation of CT-26 tumor-bearing mice

Murine colon adenocarcinoma cell line (CT-26) was purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). CT-26 cells were grown in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum, 2 units/ml penicillin-streptomycin, vitamins, 1 mM sodium pyruvate, 2 mM L-glutamine, and nonessential amino acids at in a 5% CO_2 incubator at 37℃ and 95% air. To establish the subcutaneous xenografts, six week old male Balb/c mice were subcutaneously injected with 5×10^6 CT-26 cells into the buttock of mice. All mice were carefully observed daily. All animal studies were performed according to a protocol approved by the Animal Care and Use Committee of Chonnam National University Medical School.

6. Imaging and biodistribution analysis

^{99m}Tc-HMPAO-DC ($2\text{-}5\times10^5$) was administered *intravenously* into mice. At different time points, mice images were acquired with a gamma camera (DSX-SMV, France) equipped with 1 mm pinhole collimator. The energy window was centered at 140 KeV with a 20% window. Whole body images of mice were obtained by placing the animal directly below on camera head at 1, 3, 6, 12, and 24 hours after injection of DC. Images were digitally stored in a 256×256 matrix. Image analysis was performed by using region-of-interest (ROI) analysis of the lung, liver, and spleen to obtain the decay-corrected counts. The measured activity in tissues and samples was expressed as the percent injected dose per gram tissue (%ID/g) for lungs, liver, spleen, and kidneys.

For tissue biodistribution study, five groups of mice were analyzed at different time points after injection of the ^{99m}Tc-HMPAO labeled DC. Six mice per experimental group were injected either intravenously into the lateral tail vein. Twenty four hours after cell transfer, mice were killed by cervical dislocation. The following organs were removed, weighted, and counted in a gamma counter: heart, lung, liver, spleen, kidney, bone and tumor.

7. Statistical analysis

Statistical analysis was performed with SPSS software (version 11.0). All values were expressed as mean± standard deviation (SD). Difference of mean among groups were tested by ANOVA or Student's *t*-test. The level of significant difference between experimental groups was set at $p<0.05$.

Results

1. Labeling efficiency (LE) of ^{99m}Tc-HMPAO labeled DC and image quality

LE of immature and mature DC were 60.4±5.4% and 61.8±6.7%, respectively. No difference of LE between immature and mature DC was noted ($p=0.57$). Although image quality was good, bladder activity appeared immediately after injection of ^{99m}Tc-HMPAO labeled DC. Delayed images also showed bladder as well as thyroid activities. However, no brain activity was noted. No difficulty was noted to evaluate activities in lungs, liver, and spleen.

2. MLR in mature DC before and after ^{99m}Tc-HMPAO labeling

As long as the cultures were supplemented with GM-CSF, strong MLR-stimulating activity developed in both ^{99m}Tc-HMPAO labeled and unlabeled mature DC. Incorporation of ^{3}H-thymidine into DC was not different whether they were labeled by ^{99m}Tc-HMPAO or not (Table 1).

Table 1. Counts per minute (cpm) of mixed mature dendritic cells (DC) and T cells according to number of DC per 50,000 T-cells (DC/T)

DC/T	cpm		p value
	before labeling	after labeling	
800	1,147±236	961±80	p=0.16
3,200	1,538±724	1,808±552	p=0.76
12,500	3,534±419	3,365±568	p=0.51
50,000	6,134±1,048	5,048±853	p=0.26

Table 2. Biodistribution of *intravenously* injected ^{99m}Tc-HMPAO labeled dendritic cells (DC) in control and tumor-bearing mice at 24 hours, which was determined by gamma counting of dissected organs

	Control		Tumor	
	Immature DC (n=6)	Mature DC (n=6)	Immature DC (n=5)	Mature DC (n=5)
Heart	1.57±0.34	1.37±0.24	1.93±0.41	1.42±0.36
Liver	24.46±3.1	24.13±2.49	27.07±1.91	26.03±3.82
Lung	14.35±4.26	12.54±3.91	12.13±3.01	10.72±2.39
Spleen	32.15±4.11*	38.26±3.96*	35.88±3.72*	40.44±4.14*
Kidney	16.86±3.7	14.77±2.70	15.14±1.07	14.67±2.22
Bone	2.34±0.51	2.18±0.46	2.44±0.52	2.74±0.12
Tumor	-	-	1.73±0.15*	2.35±0.32*

Biodistribtution in dissected large organs are expressed as percentage of injected dose (%ID) per gram of tissue. Each value represents mean±SD from five mice. * $p<0.05$

3. Phenotypic analysis of mature DC before and after ^{99m}Tc-HMPAO labeling

DC cultured in the presence of GM-CSF, IL-4, TNF- expressed high levels of CD11c (93.7% and 94.1% in unlabeled and labeled DC, respectively), and intermediate level of antimouse IAb (50.9% and 51.7% in unlabeled and labeled DC, respectively), and CD86 (39.4% and 42.2% in unlabeled and labeled DC, respectively), lower expression of CD80 (16.3% and 19.4% in unlabeled and labeled DC, respectively).

4. Biodistribution of ^{99m}Tc-HMPAO labeled DC

Intravenously injected ^{99m}Tc-HMPAO labeled mature and immature DC initially appeared in the lungs, followed by liver and spleen several hours later (Fig. 1). Tumors were only faintly visualized. %ID/g of tissue was not significantly different in the liver and lungs between immature and mature DC in the control and tumor-bearing mice. But %ID/g of mature DC was significantly higher in spleen than that of immature DC. Migration of mature DC to tumor was also significantly higher than that of immature DC (2.4±0.3% vs 1.7±0.2%, $p=0.03$) (Table 2).

Discussion

In this study the migration patterns of ^{99m}Tc-HMPAO labeled immature and mature DC was examined in control and tumor-bearing mice. *Intravenously* injected DCinitially accumulated in lungs, and then in spleen and liver. Interestingly, mature DC migrated more into spleen and tumor tissue than did immature DC when they were administered *intravenously*.

Several reports using ^{111}In labeled DC showed similar migration patterns to this study. Kupiec-Weglinski et al.[9] used ^{111}In-tropolone with LE of 〉65%. Radiolabeled, unsorted splenic DC were administered *intravenously* into syngeneic mice. DC were sequestered primarily in the lungs until around 1 hour, but the radioactivity declined exponentially to minimal levels by 24 hours. At the same time there was a progressive increase in the numbers of DC entering the liver and spleen, reaching plateau levels between 3 and 24 hours. Sequestrations of sorted mature DC in liver and spleen at 24 hours were 57.0% and 6.9%,

respectively. The migration of DC to mesenteric and peripheral LN, and to Peyer's patches was negligible. Therefore, the primary lymphoid site of DC localization was spleen. Although they used splenic DC, migration patterns were similar to those of this study. The same group also studied the localization of DC within the spleen using DC labeled with a fluorochrome, Hoescht 33342.[12] Spleens were removed 3 or 24 hours later and DC were visualized within particular areas that were defined by monocolonal antibodies and FITC anti-immunoglobulins. At 3 hour most DC were in the red pulp, whereas by 24 hours the majority had homed to T-dependent areas of the white pulp. Eggert et al.[13] used ^{111}In-oxine for radiolabeling of DC and resulted in a high LE of 85-90%. They also found the similar migration pattern of *intravenously* injected ^{111}In labeled DC: immediate accumulation in capillary rich, well perfused organs like the liver, lung, spleen, and kidneys. Their data indicated that ^{111}In labeled DC were well suited to follow DC migration *in vivo* and that, after *intravenous* injection, DC accumulated preferentially in the spleen. Morse et al.[14] also showed similar data to this study. They used ^{111}In-oxyquinoline labeled DC for imaging patients with metastatic malignancies. LE was 60-80%. MLR did not changed after radiolabeling as showed in this study. In three patients with *intravenously* administered ^{111}In-DC, in the first minute, the activity was localized to the lungs. Activity appeared in the liver and spleen by 1 hour. By 24 hours, the activity was predominantly localized to the liver, spleen, and bone marrow, but none was observed in any LN or tumor masses. Olasz et al.[15] developed a novel method for labeling mouse bone marrow-derived DC (BMDC) with the positron emitting radioisotope ^{18}F using N-succinimidyl-4-^{18}F-fluorobenzoate, which covalently binds to the lysine residues of cell surface proteins. Migration of ^{18}F-labeled BMDC was studied after footpad injection by ex vivo counting of dissected tissues using a gamma counter and in vivo by imaging mice with projection imager/positron emission tomography (PiPET). Quantitative assessment of cell migration by PET projection imaging of mice confirmed the ex-vivo counting results.

All of the previous studies of radiolabeled DC used mature DC. Difference of immature and mature DC in their migration after administration was not studied so far. Our current study showed that mature DC migrated more to spleen than immature DC did. Immature DC were found to express low levels of the chemokine receptor CCR7 that binds to CCL21/SLC and CCL19/ELC, present in the lymphatic vessels and lymphoid tissues.[16] When immature DC were induced to undergo maturation using such stimuli as IL-1, TNF or LPS, expression of CCR7 was up-regulated, while expression of inflammatory chemokine receptors was decreased. Functional CCR7 is necessary for the trafficking of DC into the spleen and T cell areas of lymph nodes where antigen presentation by DC to T cells occurs. Up-regulation of CCR7 in mature DC is a crucial event for the correct localization of these cells in T-cell areas. MIP-3β and secondary lymphoid tissue chemokine (SLC) are specifically expressed in T-cell-rich areas of tonsils, spleen, and LN, where mature DC home to become interdigitating DC.[17-19] The crucial role of SLC/MIP-3β and CCR7 clearly is reflected in mice deficient for these proteins. In mice homozygous for an autosomal recessive mutation, the paucity of lymph node T cells (plt), naive T cells fail to home to secondary lymphoid organs. The plt mutation is associated with a defective expression of SLC in lymphoid organs. DC from these mice fail to accumulate in the spleen and T-cell areas of lymph nodes.[20] Similarly, CCR7 knockout mice showed a defective architecture of secondary lymphoid organs and a defective homing of DC and lymphocytes.[21]

In this current study, although it is low, radioactivity of DC was found in tumor tissues and that of mature DC revealed higher than that of immature DC. We can hypothesize that small fractions of DC might migrate to tumor tissue by chemokines secreted by tumor cells. Because the expression of chemokine receptor is up-regulated in mature DC, their activity was higher in tumor tissue than that of immature DC. The localization of the different DC subsets and their fine positioning within tissues is tightly controlled by a variety of molecules regulating cell trafficking, among which chemokines have been shown to play a prominent role. In the case of cancer, tumors secrete inflammatory cytokines that induce the expression of chemokines involved in promoting the

growth of vascular tissue, providing a link to the chemokine system's role in the establishment of cancers. In particular, the CXC family of chemokine ligands, (and their corresponding receptors), have been implicated the survival, growth and migration of human cancers and in the process of tumor angiogenesis.[22]

This study has several limitations. First, migration of DC was examined only by ^{99m}Tc-HMPAO labeled DC. Comparative study using ^{111}In labeled DC was not performed. Although most of the separate studies have utilized ^{111}In oxine which is nontoxic, labels cells with high efficiency, and remains in association with cells in vivo, ^{99m}Tc labeled agents have favorable physical characteristics and several advantages for gamma camera imaging. They permit rapid imaging, good image resolution and readily available in routine clinical setting for all nuclear medicine laboratories because of a product of generator system.It is significantly less expensive than ^{111}In and is always available. HMPAO is a small, neutral, and lipophilic complex that binds ^{99m}Tc. The lipophilicity of HMPAO allows it to cross the leukocyte cell membrane, the primary ^{99m}Tc-HMPAO complex changes into a hydrophilic secondary complex that is trapped within the cell. The radioactivity is bound to intracellular organelles, primarily the mitochondria and nucleus. Most studies using ^{111}In labeled or ^{99m}Tc-HMPAO labeled cells showed similar results.[23] Second, our results with intravenous ^{99m}Tc-HMPAO labeled DC administration showed immediate appearance of bladder suggesting fast metabolization of released ^{99m}Tc-HMPAO. In a separate study,[24] significant early bladder activity was also observed as demonstrated in this study. This indicates that the activity was released from DC as a labeled molecule different from lipophilic ^{99m}Tc-HMPAO. This is confirmed by the absence of brain

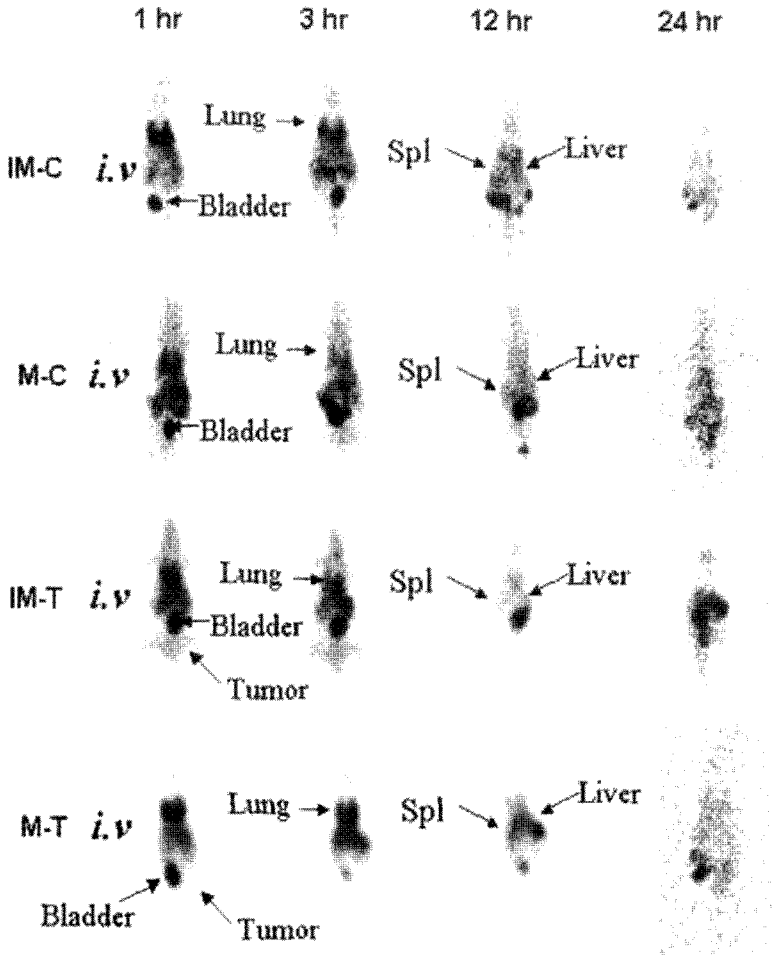

Fig. 1. Biodistribution of intravenously (i.v.) administered ^{99m}Tc-HMPAO labeled dendritic cells (DC) at 1, 3, 12, and 24 hours after injection. DC are initially accumulated in lungs, then migrate to liver and spleen. Bladder activity is visualized from early images. Mature DC migrate into spleen and tumor slightly more than immature DC. IM-C: immature DC in non-tumor-bearing control mice, M-C: mature DC in non-tumor-bearing control mice, IM-T: immature DC in tumor-bearing mice, M-T: mature DC in tumor-bearing mice, Spl: spleen.

activity. Rapid renal elimination of the radioactivity reflects the fast release of a hydrophilic ^{99m}Tc labeled compounds from DC, probably resulting from lysosomal processing of the ^{99m}Tc-HMPAO initially used for labeling. Therefore more valid method of radiolabeling DC with ^{99m}Tc should be further investigated. Finally, resolution of gamma camera imaging is low to see differences of radioactivities in lymphoid organs. However, our current study exhibited the clearance of DC from the lungs (Fig. 1) under the radionuclide imaging and revealed later accumulation in spleen using biodistribution study. Experimental design using advanced techniques with higher resolution and specificity is recommended to solve this problem.

In conclusion, both mature and immature DC of mice were successfully radiolabeled by ^{99m}Tc-HMPAO. They kept immunologic function after ^{99m}Tc-HMPAO labeling. Mature DC migrated more into spleen and tumor tissue than immature DC when they were injected *intravenously*.

요 약

목적: 이 연구는 ^{99m}Tc-HMPAO에 표지된 미성숙 또는 성숙 수지상 세포의 마우스 생체 내 분포와 이동 양상에 대해 알아보고자 하였다. **대상 및 방법:** 마우스의 대퇴골과 경골의 골수로부터 수지상 세포를 배양하고 미성숙, 성숙 수지상세포를 ^{99m}Tc-HMPAO로 표지하였다. 방사성 표지 전후에 수지상 세포의 기능 및 표현형의 변화 유무를 알기 위해 동종 혼합 림프구 반응 (allogeneic mixed lymphocyte reaction)과 형광 활성 세포 선별(fluorescence-activated cell sorting)을 시행하였다. 정맥 주사된 수지상 세포의 생체 내 이동은 감마 카메라 영상과 생체 분포 실험을 통하여 평가하였고, 피하 종양 마우스 모델과 대조군에서 비교하였다. 폐, 간, 비장, 신장, 종양 등 조직에서 그램 당 주사량의 백분율(%ID/g)을 계산하였다. **결과:** 미성숙, 성숙 수지상 세포의 표지 효율은 각각 60.4±5.4%와 61.8±6.7% 였다. 수지상 세포의 정맥주사 후 방사능은 폐에서 가장 먼저 관찰되었고, 이후 간과 비장에 분포되었다. 성숙 수지상 세포가 미성숙 수지상 세포에 비해 비장으로 더 많이 이동하였다(대조군: 38.3±4.0% vs. 32.2±4.1%, 종양이식 군: 40.4±4.1% vs. 35.9±3.8%, $p<0.05$). 종양으로의 이동 역시 성숙 수지상 세포가 미성숙 수지상 세포에 비해 더 많은 비율을 보였다(2.4±0.3% vs 1.7±0.2%; $p=0.034$). **결론:** ^{99m}Tc-HMPAO 에 표지된 수지상 세포를 이용하여 마우스 생체 내 이동을 실시간 영상화 할 수 있었다. 마우스 정맥에 주사되었을 때, 더 많은 비율의 성숙 수지상 세포가 미성숙 수지상 세포에 비해서 비장이나 종양으로 이동함을 알 수 있었다.

References

1. Caux C, Ait-Yahia S, Chemin K, de Bouteiller O, Dieu-Nosjean MC, Homey B, et al. Dendritic cell biology and regulation of dendritic cell trafficking by chemokines. *Springer Semin Immunopathol* 2000;22:345-69.
2. McAfee JG, MacVittie TJ. The impact of recent advances in immunology and cancer therapy on nuclear medicine. *Semin Nucl Med* 2001;31:342-9.
3. Allavena P, Sica A, Vecchi A, Locati M, Sozzani S, Mantovani A. The chemokine receptor switch paradigm and dendritic cell migration: its significance in tumor tissues. *Immunol Rev* 2000;177:141-9.
4. Murphy G, Tjoa B, Ragde H, Kenny G, Boynton A. Phase I clinical trial: T-cell therapy for prostate cancer using autologous dendritic cells pulsed with HLA-A0201-specific peptides from prostate-specific membrane antigen. *Prostate* 1996;29:371-80.
5. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabble S, Dummer R, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nature Med* 1998;4:328-32.
6. Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, et al. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nature Med* 1996;2:52-8.
7. Bender A, Sapp M, Schuler G, Steinman R, Bhardwaj N. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J Immuno Methods* 1996;196:121-35.
8. Kung HF, Ohmomo Y, Kung MP. Current and future radiopharamaceuticals for brain imaging with single photon emission computed tomography. *Semin Nuc Med* 1990;2:290-302.
9. Kupiec-Weglinski JW, Austyn JM, Morris PJ. Migration patterns of dendritic cells in the mouse. Traffic from the blood, and T cell-dependent and -independent entry to lymphoid tissues. *J Exp Med* 1988;167:632-45.
10. Garrigan K, Moroni-Rawson P, McMurray C, Hermans I, Abernethy N, Watson J, et al. Functional comparison of spleen dendritic cells and dendritic cells cultured in vitro from bone marrow precursors. *Blood* 1996;88:3508-12.
11. Godfrey WR, Ge YG, Spoden DJ, Levine BL, June CH, Blazar BR, et al. In vitro-expanded human CD4(+)CD25(+) T-regulatory cells can markedly inhibit allogeneic dendritic cell-stimulated MLR cultures. *Blood* 2004;104:453-61.
12. Austyn JM, Kupiec-Weglinski JW, Hankins DF, Morris PJ. Migration patterns of dendritic cells in the mouse. Homing to T cell-dependent areas of spleen, and binding within marginal zone. *J Exp Med* 1988;167:646-51.
13. Eggert AA, Schreurs MW, Boerman OC, Oyen WJ, de Boer AJ, Punt CJ, et al. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. *Cancer Res* 1999;59:3340-5.
14. Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK. Migration of human dendritic cells after injection in patients with metastatic malignancies. *Cancer Res* 1999;59:56-8.
15. Olasz EB, Lang L, Seidel J, Green MV, Eckelman WC, Katz SI. Fluorine-18 labeled mouse bone marrow-derived dendritic cells can be detected in vivo by high resolution projection imaging. *J Immunol Methods* 2002;260:137-48.
16. Cravens PD, Lipsky PE. Dendritic cells, chemokine receptors and

autoimmune inflammatory diseases. ***Immunol Cell Biol*** 2002;80: 497-505.
17. Dieu MC, Vandervliet B, Vicari A, Bridon JM, Oldham E, Ait-Yahia S, et al. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. ***J Exp Med*** 1998;188:373-86.
18. Ngo VN, Tang HL, Cyster JG. Epstein-Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naive T cells and activated B cells. ***J Exp Med*** 1998;188:181-91.
19. Willimann K, Legler DF, Loetscher M, Stuber Roos R, Delgado MB, Clark-Lewis I, et al. The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7. ***Eur J Immunol*** 1998; 28:2025-34.
20. Gunn MD, Kyuwa S, Tam C, Kakiuchi T, Matsuzawa A, Williams LT, Nakano H. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. ***J Exp Med*** 1999;189:451-60.
21. Frster R, Schubel A, Breitfeld D, Kremmer E, Renner-Mller I, Wolf E, Lipp M. CCR7 coordinates the primary immune response by establishing functional microenviroments in secondary lymphoid organs. ***Cell*** 1999;99:23-33.
22. Vicari AP, Treilleux I, Lebecque S. Regulation of the trafficking of tumour-infiltrating dendritic cells by chemokines. ***Semin Cancer Biol*** 2004;14:161-9.
23. Costa DC, Lui D, Ell PJ. White cells radiolabelled with 111In and 99Tcm--a study of relative sensitivity and in vivo viability. ***Nucl Med Commun*** 1988;9:725-31.
24. Blocklet D, Toungouz M, Kiss R, Lambermont M, Velu T, Duriau D, et al. ^{111}In-oxine and ^{99m}Tc-HMPAO labelling of antigen-loaded dendritic cells: in vivo imaging and influence on motility and actin content. ***Eur J Nucl Med Mol Imaging*** 2003;30:440-7.