Perfluoro-15-crown-5 ether-labeled dendritic cells

PFPE-DCs

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Chemical name:	Perfluoro-15-crown-5 ether-labeled dendritic cells	
Abbreviated name:	PFPE-DCs	
Synonym:		
Agent category:	Labeled cell	
Target:	Other	
Target category:	Other –inflamed tissue	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	^{19}F	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Dendritic cells (DCs), known as antigen-presenting cells, are found in almost all peripheral tissues and in primary/secondary lymphoid organs (1). DCs are the initiator and modulator in the adaptive immune responses against bacteria, viruses, allergens, and tumor antigens (2). DCs in peripheral tissues are responsible for the capture of antigens (3). In the absence of inflammation, DCs remain in an immature state. The captured antigens are transported with DCs to the lymph node, but no co-stimulatory activation to T cells occurs. In the presence of inflammation, numerous mature DCs migrate to the draining lymph nodes. During migration, the antigens are processed into small peptides

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bound to the major histocompatibility complex (MHC) on the surface of the DCs. At the lymph nodes, mature DCs present the MHC-peptide complex to naïve T cells ($CD4^+$ T helper cells and cytolytic $CD8^+$ T cells) to activate them. After activation, a series of immune responses are completed through the interactions of T cells with other cells and molecules such as B cells for antibody formation, macrophages for cytokine releases, and targets for lysis (1). Behaving as mobile sentinels, DCs bring antigens to T cells and express co-stimulators to induce immunity. DCs have been used in many clinical trials to treat cancers and immunological disorders (4). Because the migratory properties of DCs are directly related to their function (5), non-invasive tracking of DCs becomes very important to clinic applications (2).

Being the only stable isotope of fluorine, with a natural abundance of ~100%, ^{19}F has a nuclear spin 1/2 with a large gyromagnetic ratio (γ ~40.05 MHz/T) (6). The small γ difference between the ^{19}F and ^{1}H (~6%) allows the use of existing proton nuclear magnetic resonance (NMR) instrumentation with minor adjustments to detect fluorinated species at high sensitivity (~83% relative to ^{1}H). Endogenous fluorine *in vivo* is found primarily in bones and teeth as solid fluorides, which have very short T_2 relaxation times and result in an undetectable signal with NMR imaging. Therefore, exogenously administered fluorinated tracers can be used to track various biological processes *in vivo*. For example, perfluorocarbons (PFCs) are used to measure oxygen tensions in tissues and tumors (7). The lack of background ^{19}F signal is advantageous in *in vivo* applications, but additional ^{1}H images are required to provide anatomic interpretations. PFCs are extremely hydrophobic and do not dissolve in blood directly; they normally are formulated as biocompatible emulsions for intravenous administration (6). Inside the body, the PFC particles are cleared from circulation by phagocytes/ macrophages or by respiration within several hours or days, depending on the administered dose, particle size, and PFCs (8). Many commercial PFC emulsions have been found to be nontoxic or do not cause any health problems other than tissue swelling (6). Perfluoro-15-crown-5 ether (perfluoropolyether, PFPE), a commonly used PFC, contains 20 equivalent ^{19}F spins that generate a single resonance (-92.5 ppm) in NMR imaging (9). This singlet simplifies its images such that no chemical shift–induced artifact is expected. PFPE can be emulsified to form particles of ~100–200 nm in diameter, allowing for cellular uptake *via* endocytosis (4). DCs are labeled with PFPE (PFPE-DCs) for ^{19}F magnetic resonance imaging (MRI).

Synthesis

[PubMed]

Ahrens et al. briefly described the detailed preparation of PFPE-DCs (4). First, commercially available PFPE was emulsified (40% vol/vol) in a mixture of H_2O, 2% lecithin, and 2% safflower oil to produce PFPE nanoparticles ~100–200 nm in diameter. Two types of DCs were used for labeling: a fetal skin-derived DC line (FSDCs) and primary cells derived from bone marrow (BMDCs). For FSDCs, the cells were labeled by incubating PFPE emulsion (2 µl/ml) with dendritic cells for 18 h at 37 °C. The labeled cells

(PFPE-FSDCs) were then suspended in solution. Adding transfection agents such as lipofectamine enhanced the labeling efficiency up to 26-fold with the same incubation time. For BMDCs, PFPE emulsion (2 µl/ml) was added to the bone marrow culture and incubated for 18 h at 37 °C. The harvested PFPE-labeled BMDCs (PFPE-BMDCs) were then incubated on a poly-L-lysine–coated plate for 1 h at 37 °C to adhere cells. Dead and non-adherent cells were discarded. The adherent cells were removed with trypsin-EDTA treatment to yield a BMDC purity of 80–90% as determined with flow cytometry for the expression of DC marker CD11c. The amount of PFPE was found to be 5.2×10^{12} fluorine spins (~0.25 ng PFPE) per FSDC or BMDC as determined with ^{19}F NMR, allowing for detection of ~2×10^5 labeled cells per voxel.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Ahrens et al. used ^{19}F NMR to examine the intracellular retention of the PFPE in DCs *in vitro* (4). The ^{19}F signal for PFPE-FSDCs was monitored with an 11.7-T (^{19}F resonant frequency 470 MHz) NMR spectrometer for 5 d. The cell division caused dilution that led to a decrease in ^{19}F signal; ~15% signal was observed after 5 d. During this time, the PFPE appeared to remain stable in that no change was found in its spectral line shape. The cellular internalization of PFPE emulsion particles was examined with fluorescence microscopy and transmission electron microscopy. FSDCs were fluorescence-labeled with fluorescein isothiocyanate-dextran (FITC-DX). The PFPE was also tagged with a fluorescent probe, lipophilic dialkycarbocyanine (DiI) fluorophore, in the presence of lipofectamine. Then the fluorescence-labeled FSDCs were incubated with the fluorescence-labeled PFPE for 18 h at 37 °C as before. Under an electron microscope, the intracellular PFPE particles appeared as numerous, bright, smooth spheroids located within the macropinosomes, which are large vesicular compartments containing fluorescein. The osmium staining used to visualize intracellular structures also demonstrated the presence of unsaturated lipids in the particle's surfactant. Some PFPE particles were clustered together or wrapped with surrounded multiple membrane compartments relating to MHC class II–enriched compartments.

Ahrens et al. examined the effects of PFPE on cellular toxicity, proliferation, metabolism, and phenotype of labeled DCs were examined *in vitro* (4). The cytotoxicity of PFPE was evaluated with an assay that measured the leakage of the enzyme glucose 6-phosphate dehydrogenase (G6PD) from the cytoplasm into the culture medium. Little or no apparent cytotoxicity was found for all conditions studied. The cellular proliferation was assayed with methyl thiazole tetrazolium (MTT) and the total double-stranded DNA (dsDNA), which was further confirmed by direct cell counts. No apparent difference in cell proliferation was found between labeled cells and controls. The effect on cellular phenotype was examined *via* flow cytometric analysis of cellular markers. Several surface markers in the labeled DCs were compared with those in the unlabeled DCs, including the co-stimulatory molecule CD80 and MHC class II. The presence of PFPE emulsion

particles or the transfection agent lipofectamine did not appear to induce maturation of DCs.

Animal Studies

Rodents

[PubMed]

Ahrens et al. examined the migration of PFPE-DCs in mice with *in vivo* ^{19}F MRI/^{1}H MRI (4). Mice were injected with 8 × 10^6 PFPE-FSDCs intramuscularly. T_2-Weighted ^{1}H images and ^{19}F images were collected with an 11.7-T imager 8 h after the injection. The hyperintense sites in the ^{1}H images, which reflected the presence of inflammatory cells or excess phosphates, appeared to correspond to sites with strong signals in ^{19}F images. From these sites, the ^{19}F signals were tracked farther away as a sign of DC migration to the draining lymph node. Ahrens et al. then studied the migration of PFPE-BMDCs from the foot to the lymph nodes (4). Mice received 4 × 10^6 PFPE-BMDCs *via* subcutaneous injection into the tip of the hind footpad. ^{19}F/^{1}H images collected 6 h after injection exhibited a strong ^{19}F signal (signal/noise ratio >10) in the popliteal lymph node located adjacent to the knee, which suggests that the cells migrate towards the lymph node. To confirm this migration pattern, the BMDCs were double-labeled with PFPE and 5-chloromethylfluorescein diacetate. Control mice were injected in one foot with the double-labeled BMDCs and in the other foot with PFPE-BMDCs. The efferent popliteal node and the inguinal/axillary nodes as controls were excised 24 h after injection. Single-cell suspensions were prepared from these excised tissues and analyzed with flow cytometry. Fluorescent cells were visible in the popliteal lymph nodes of mice receiving double-labeled BMDCs but not in mice that received PFPE-BMDCs. The biodistribution of PFPE-FSDCs was examined in mice with *in vivo* ^{19}F MRI/^{1}H MRI (4). Mice received an intravenous injection of 18 × 10^6 PFPE-FSDCs, and ^{19}F/^{1}H images were collected after inoculation. A strong ^{19}F signal (signal/noise ratio ~9) was found in the liver and spleen, and a weak signal was observed in the lung.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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