Perfluoropolyethylene glycol–labeled BDC2.5 T cells

PFPE-BTCs

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Chemical name:	Perfluoropolyethylene glycol–labeled BDC2.5 T cells	
Abbreviated name:	PFPE-BTCs	
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]

T cells (TCs) are responsible for regulating immune responses and maintaining immune tolerance *via* recognition of peptide antigens that are bound to human leukocytes (1). Some TCs possess autoimmunity or self-tolerance through recognition of self-antigens. Loss of this required self-tolerance can result in an autoimmune disorder. For example, type 1 diabetes (T1D) is characterized by a spontaneous loss of immunological tolerance of pancreatic β-cells, which leads TCs to attack insulin-producing β-cells in the islets of Langerhans in the pancreas (2). The development of T1D has two stages: the insulitis phase when mixed leukocytes invade the islets and destroy β-cells, and the diabetes phase

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when the bulk of β-cells (up to 90%) have been destroyed and no longer produce sufficient insulin to control blood glucose levels (2). These stages can be reproduced easily in the non-obese diabetic (NOD) mouse model for human T1D (3), a spontaneous murine model in which insulitis and diabetes appear mainly in females in ~5 and ~13 weeks, respectively (4). Studies in NOD mice demonstrate that the islets in the pancreas are infiltrated with autoreactive $CD4^+$ TCs as majority cells, as well as other cells such as $CD8^+$ TCs, dendritic cells, B cells, and macrophages, in the insulitis phase (3). From the spleen and lymph nodes of female NOD mice, islet antigen-specific TCs, known as BDC2.5 TCs (BTCs), are derived and identified to be diabetogenic $CD4^+$ TCs (5). Adoptive transfer of BTCs to NOD severe combined immunodeficiency (SCID) mice can accelerate diabetes (6). Expressing TC receptor genes (TCR) of BTCs in transgenic (Tg) mice produce a murine model (BDC2.5 TCR Tg/NOD SCID mice) with monoclonal TC repertoire in which all of the TCs are $CD4^+$ type (5). Imaging these TCs in the progression of T1D is important to the understanding of the pathogenesis of autoimmunity and to the design of immunotherapeutic interventions (6).

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) (7). 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 magnetic resonance imaging (MRI). Therefore, exogenously administered fluorinated tracers can be used to track various biological processes *in vivo*. For example, perfluorocarbons (PFC) such as perfluoro-15-crown-5 (15C5) have been used to measure oxygen tensions in tissues and tumors (8) and to label dendritic cells (9). 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 (7). Inside the body, the PFC particles are cleared from circulation by phagocytes/macrophages and/or by respiration within several hours or days, depending on the administered dose, particle size, and PFC (10). Many commercial PFC emulsions have been found to be nontoxic or do not cause any health problems other than tissue swelling (7). Perfluoropolyethylene glycol (PFPE, Mw 1750) is a linear PFC that contains a large number of ^{19}F atoms per molecule for enhancing sensitivity (6). The center CF_2 groups can generate a strong peak (>0.9 at -92 ppm) that dominates 90% of its ^{19}F signal, whereas the end CF_2 groups yield a weak signal (<0.1 at -79 ppm) that is below the *in vivo* MRI detection limit. The spin relaxation time (T_1) is 2.2 times shorter than that of 15C5 at 11.7 T, which allows a shorter imaging acquisition time. PFPE can be emulsified to form particles ~100–200 nm in diameter, allowing for cellular uptake *via* endocytosis (9). BTCs are labeled with PFPE (PFPE-BTCs) for ^{19}F MRI.

Synthesis

[PubMed]

Srinivas et al. briefly described the detailed preparation of PFPE-BTCs (6). First, commercially available PFPE was emulsified with commercially available surfactant poloxamers at a molar ratio of 1:1 to produce PFPE nanoparticles 103 ± 4 nm in diameter. Poloxamers are nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene flanked by hydrophilic chains of polyoxyethylene. TCs derived from the BDC2.5 TCR Tg mouse were activated *in vitro* in the presence of 1 μg/mL anti-CD28 and 10 U/mL IL-2, followed by incubation with PFPE emulsion and the commercial transfection agent FuGEN 6 for 3.5 h at 37°C to yield PFPE-BTCs. PFPE was found to exhibit 2.2×10^{13} fluorine spins per TC as determined by ^{19}F NMR. The chemical shifts were found to be -92 ppm for the major peak (central CF_2 groups) and -79 ppm for the minor peak (end CF_2 group).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The effects of PFPE on cytotoxicity, cellular viability, and phenotype of labeled BTCs were examined *in vitro* (6). A Trypan blue exclusion assay was used to evaluate the cytotoxicity of PFPE. No apparent cytotoxicity was found for all conditions studied. A methyl thiazole tetrazolium assay was used to determine the cellular viability, which was ~95% 2–48 h after labeling. Flow cytometry was used to assess several cellular surface markers in the BTCs, including CD62L (a lectin-binding protein) and CD4 (a major histocompatibility complex (MHC) II-interacting co-receptor). No apparent downregulation was found in the expression of CD62L in the labeled cells compared with that in the unlabeled cells, which suggests that the labeling with PFPE emulsions did not appear to activate naïve TCs. A transient reduction of CD4 expression was observed in the presence of transfections agents, which was related to the receptor-mediated endocytosis of PFPE nanoparticles. The cellular internalization was examined with PFPE emulsions labeled with the fluorescent probe dialkylcarbocyanine dye (DiI) *via* fluorescence microscopy. The fluorescence-labeled PFPE appeared to bind to the cell membrane or to be localized within the cells.

Animal Studies

Rodents

[PubMed]

Srinivas et al. examined the migration of PFPE-BTCs in mice with *in vivo* ^{19}F MRI/^{1}H MRI (6). NOD SCID mice (8–10 weeks old, $n = 4$) received intraperitoneal injections of 4×10^6 PFPE-BTCs and imaged 48 h later with an 11.7-T (^{19}F resonant frequency 470 MHz) NMR spectrometer. The ^{19}F images demonstrated a localized signal in the region

of the pancreas but not in the liver or spleen. This result was further confirmed with histological analysis of pancreas. TCs were found around the islet in the pancreas 24 h after adoptive transfer of PFPE-BTCs, suggesting that the labeled TCs were capable of homing to the pancreas *in vivo*. From the *in vivo* ^{19}F images, the number of detected TCs in the pancreas was quantified as 1.5% to 3.4% of the total transferred cells with the average cell density of ~28,000 cells per voxel, which was verified to be 2.9 ± 0.3% with *ex vivo* ^{19}F NMR. The majority of transferred cells remained at the injection site or circulating in the body, and their concentrations were too low to be detected with *in vivo* ^{19}F MRI. As controls, PFPE emulsions or labeled non-specific TCs (MHC-mismatch non-specific TCs derived from MHC-mismatched BLAB/c mice) were intraperitoneally injected into NOD SCID mice, and the ^{19}F images were collected 48 h later. For PFPE emulsions, some ^{19}F signal was found in the abdominal cavity near the bladder but not in pancreas. For the non-specific TCs, no ^{19}F signal was found in or around the pancreas.

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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