Cy5.5-CGRRRQRRKKRG-Labeled T lymphocytes

Cy5.5-Tat-T cells

Huiming Zhang, PhD[1]

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Chemical name:	Cy5.5-CGRRRQRRKKRG-Labeled T lymphocytes	
Abbreviated name:	Cy5.5-Tat-T cells	
Synonym:		
Agent category:	Labeled cell	
Target:	Other	
Target category:	Other –inflamed tissue	
Method of detection:	Near-infrared fluorescence (NIRF) optical imaging	
Source of signal/contrast:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

T cells are responsible for regulating immune responses and maintaining immune tolerance *via* recognition of peptide antigens that are bound to human leukocytes (1). Some T cells possess autoimmunity or self-tolerance through recognition of self-antigens. Loss of this required self-tolerance can result in an autoimmune disorder. For instance, experimental allergic encephalomyelitis (EAE) is one of immune-mediated diseases in which immune cells become reactive against myelins, which leads to the destruction of myelin sheets (2). EAE can be induced in rodents by adoptive transfer of $CD4^+$ T cells specific to myelin basic protein (MBP), an autoantigen of myelin (3). As an animal model for the inflammatory disease, EAE can reproduce many clinical neuropathological and immunological aspects of multiple sclerosis (MS) and thus has been widely used in

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

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therapeutic development for MS (4). The evolution of inflammatory lesions in EAE involves several steps (1, 3). After intravenous administration, the injected MBP-specific T cells cross the blood–brain barrier to recognize the T cell antigen located on perivascular microglia. This antigen-specific interaction produces a plethora of inflammatory cytokines and mediators, leading to amplification of the inflammatory reaction. The blood–brain barrier then opens to allow antigen-independent recruitment of various mononuclear inflammatory cells into the central nervous system, including additional T cells, macrophages, and granulocytes. Consequently, the inflammatory lesions evolve into severe neurological dysfunctions such as ascending paraparesis and paralysis. Because the activated T cells are involved in the entire process, the trafficking of the activated MBP-specific T cells in EAE can reflect their immune activity in every evolutionary phase (2). Labeling T cells with imaging probes will allow non-invasive tracking of the migration of T cells *in vivo*.

The imaging probes can be internalized into cells with the use of cell-penetrating peptides (CPP) as vector/nuclear delivery vehicles of conjugated cargo (5). In general, CPP comprise a protein-transduction domain formed by small peptides (<20 amino acids) for cell membrane translocation. A commonly used CPP is a peptide truncated from the 86-mer transactivating transcriptional activator (Tat) protein in human immunodeficiency virus type 1 (HIV) (6). In particular, Tat(47-57) is widely used in cellular delivery of peptides, proteins, genetic material, antibodies, nanoparticles, and liposomes (5). Tat(47-57) contains an α-helical structure with a charged face formed by six arginine and two lysine residues (6). Tat-mediated internalization consists of multiple steps: the binding of Tat to the cell surface, stimulation of macropinocytotic uptake of Tat and transfer into macropinosomes, and finally endosomal escape into the cytoplasm (7).

A T lymphocyte is labeled with Cy5.5-Cys-Gly-Arg-Arg-Arg-Gln-Arg-Arg-Lys-Lys-Arg-Gly (CGRRRQRRKKRG) (Cy5.5-Tat-T cell) for optical imaging (2). This agent contains a near-infrared fluorescence (NIRF) dye shuttled across the cell membrane of T cells *via* Tat(48-57). The fluorescence probe Cy5.5 is a cyanine dye consisting of two quaternized heteroaromatic bases (A and A') joined by a polymethine chain with five carbons (8), and it is bound to cysteine-terminated Tat(48-57) *via* a maleimide group as a spacer (2). Cy5.5 has a delocalized positive charge in its chromophore and possesses high quantum yield (0.22 at 678 nm), good chemical stability, easy conjugation, and high sensitivity (mole extinction coefficient ~250,000 mol/cm) (9, 10). The excitation/emission wavelength is 674/692 nm for Cy5.5, where hemoglobin and water have their lowest absorption coefficient. The labeled cargo Cy5.5-Tat is translocated through the plasma membrane of cells by an energy-dependent process involving endocytosis (2). The produced Cy5.5-Tat-T cells can be adoptively transferred to EAE rats for visualization and quantification of T cells activity in the early inflammation stages of EAE.

Synthesis

[PubMed]

Berger et al. briefly described the detailed preparation of Cy5.5-Tat-T cells (2). A cysteine-terminated Tat peptide (48-57) derived from 86-mer Tat protein (CGRRRQRRKKRG) was coupled with Cy5.5-maleimide (Cy5.5-Mal) in dimethyl sulfoxide at a ratio of 1:1. Then T cells were incubated with Cy5.5-Tat (molecular weight, 2596.1) at a concentration of 5–200 μg/ml overnight. The amount of Cy5.5-Tat peptide was estimated to be 5–50 pg per T cell as determined with *in vitro* measurement of Cy5.5 fluorescence. Cy5.5-Tat was found to be located predominantly in the cytoplasmic compartment of T cells.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Berger et al. examined cell variability of Cy5.5-Tat-T cells in different culture media with fluorescent measurement (2). For media with 1–50 μg/ml of Cy5.5-Tat, the cell viability was not affected; however, for media with 100 and 200 μg/ml of Cy5.5-Tat, the surviving cells decreased by 10% to 30%, respectively. Berger et al. also performed a kinetic study on the labeling efficiency. T cells were incubated with 200 μg/ml Cy5.5-Tat for different durations. Incubation for 1 h generated ~19.5% labeled living cells. This fraction increased to 36.4% with 12 h of incubation, including 12.7% of $CD4^+$ and 23.7% of $CD8^+$ cells. Even with such a long incubation time, approximately one third of the total T cells were not labeled. These cells either did not favor Tat peptide mediation of cell penetration or the labels were degraded in these cells.

Animal Studies

Rodents

[PubMed]

Berger et al. used *in vivo* NIRF imaging to study the filtration of Cy5.5-Tat-T cells into the central nervous systems of EAE rats (2). Lewis rats with chronic relapsing EAE were used in the study to provide different patterns of neurological impairment during the acute phase (10–12 days postimmunization (dpi) against myelin), the remitting phase (15–20 dpi), and first relapse phase (21–26 dpi). Activated Cy5.5-Tat-T cells (~10^7 cells) were adoptively transferred intravenously at 5, 7, and 11 dpi followed by NIFR imaging up to 22 dpi. In all rats, an intense fluorescence signal was found in the brain immediately after injection of the activated Cy5.5-Tat-T cells. Pronounced variations in the different phases of the disease were observed; i.e., EAE rats injected with activated Cy5.5-Tat-T cells at 7 dpi demonstrated an increase in fluorescence before the onset of symptoms (9 dpi): the strongest intensity (~90% of its initial value) was observed at the end of the acute phase (15 dpi); a decrease to 32% was observed at remitting phase (19 dpi); and an increase to 50% was observed at relapsing phase (22 dpi). This alteration reflected the accumulation of activated Cy5.5-Tat-T cells in the inflammation sites, which triggered the infiltration of activated monocytes generated at the lesions and increased the recruitment for activated Cy5.5-Tat-T cells in the immune reaction against myelin. As controls, healthy rats

received the activated Cy5.5-Tat-T cells, naïve Cy5.5-Tat-T cells, or Cy5.5-Tat. An intense fluorescence signal was also found in the brain immediately after injection in these rats, but the intensity decreased to baseline level within 1 day and remained constant at much lower intensity levels at all other time points. Only the healthy rats injected with activated Cy5.5-Tat-T cells at 7 dpi had intensity of ~50% of its initial value during 12–15 dpi, which decreased subsequently to 32%.

Berger et al. confirmed the presence of Cy5.5-Tat-T cells in the lesions of EAE rats *ex vivo* (2). Fluorescence microscopy was used to examine the excised brain slices at 12 dpi and 22 dpi. Significant amounts of Cy5.5 were found in all EAE rats and in the healthy rats that were injected with activated Cy5.5-Tat-T cells. As controls, EAE rats injected with non-activated Cy5.5-Tat-T cells or healthy rats injected with activated Cy5.5-Tat-T cells exhibited significantly weaker fluorescence or absence of fluorescence. The slices from EAE rats were further examined with immunohistochemical stains to identify T lymphocytes with CD3 tracers and macrophages with ED2 tracers (2). The exact co-localization of CD3 and ED2 tracers in the periphery of the medulla confirmed the predominant infiltration of these cells into EAE lesions. Confocal images demonstrated that both the T lymphocytes and macrophages were labeled with Cy5.5-Tat, indicating the phagocytosis of Cy5.5-Tat-T cells by activated monocytes.

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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