CLIO-(H-2K^{d})-Lys-Tyr-Asp-Lys-Ala-Asp-Val-Phe-Leu

CLIO-NRP-V7

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Created: August 15, 2008; Updated: September 16, 2008.

Chemical name:	CLIO-(H-2K^{d})-Lys-Tyr-Asp-Lys-Ala-Asp-Val-Phe-Leu	
Abbreviated name:	CLIO-NPR-V7	
Synonym:	MN-NRP-V7	
Agent category:	Peptide, protein (nanoparticle)	
Target:	H-2K^{d}-restricted β cell-specific T cell receptor (8.3-TCR)	
Target category:	receptor	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Iron oxides	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is Currently available in PubChem.

Background

[PubMed]

Type 1 diabetes (T1D) is an organ-specific autoimmune disease originating from the destruction of pancreatic β cells by autoreactive T cells (TCs) (1). In particular, islet-associated CD8^{+} TCs initiate β cell insult and trigger shedding of β cell autoantigens. This subset of CD8^{+} TCs is primarily restricted to the class I major histocompatibility complex (MHC) molecule H-2K^{d} (2), in which Vα17-Jα42 TC receptor α (TCR-α) chains are expressed for recognition of a unique β cell autoantigen (1), islet-specific glycose-6-phosphatase catalytic subunit-related protein (IGRP). After initiation, a series of immune responses occur, including loading autoantigens into antigen-presenting cells (the

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NLM Citation: Zhang H. CLIO-(H-2K^{d})-Lys-Tyr-Asp-Lys-Ala-Asp-Val-Phe-Leu. 2008 Aug 15 [Updated 2008 Sep 16]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

dendritic cells), activating autoreactive $CD4^+$ TCs, and infiltrating TCs in pancreatic islets. T1D development consists of an insulitis phase and a diabetes phase, which are readily reproduced in a murine model called non-obese diabetic (NOD) mouse for human T1D (3, 4). In general, the progression from insulitis to diabetes in NOD mice is accompanied by alternatively repeated circulation of $IGRP_{206-214}$–reactive $CD8^+$ TC pool and avidity maturation of its islet-associated counterpart (5). Thus, IGRP has been used as a target for human diabetogenic response. NOD peptide (NRP-V7) (Lys-Tyr-Asp-Lys-Ala-Asp-Val-Phe-Leu (KYNKANVEL)) is a mimotope of IGRP that shares a 67% sequence homology with the endogenous $IGRP_{206-214}$ epitope and has high avidity for $H\text{-}2K^d$ molecule (5). A transgenic mouse (8.3-NOD) that specifically expresses $H\text{-}2K^d$–restricted TCR (8.3-TCR) has been used for a variety of therapeutic studies (6), in which 8.3-TCR constitutes as much as 30% to 40% of islet-associated $CD8^+$ TCs (2).

Cross-linked iron oxide-($H\text{-}2K^d$)-(CLIO-NPR-V7) is a NRP-V7 labeled paramagnetic iron oxide nanoparticle that is used to image 8.3-TCR by magnetic resonance imaging (MRI) (1, 5). This agent consists of three components: an iron oxide nanoparticle core for MRI contrast enhancement, two avidin molecules are covalently linked to the core surface, and eight biotinylated peptide/MHC complex (NRP-V7/$H\text{-}2K^d$) monomers bind on the avidins for recognition of 8.3-TCR. The nanoparticle contains an icosahedral core of superparamagnetic crystalline Fe_3O_4 (magnetite) that is caged by epichlorohydrin cross-linked dextran and functionalized with amine groups (CLIO-NH_2) (7). They have a high magnetic susceptibility to induce a significant magnetization inside a magnetic field. This creates microscopic field gradients that diphase nearby protons and causes a shortening of T_2 relaxation times (8). Avidin is a tetrameric protein with a molecular mass of 68 KDa that is capable of strongly binding four biotins (association constant (K_a) = 1.7×10^{15} M^{-1}) (9). Because CLIO-NRP-V7 binds to the peripheral 8.3-TCR in the blood directly, it can be used to label islet-associated TCs for *in vivo* tracking through systemic administration (1, 5).

Synthesis

[PubMed]Moore et al. described the synthesis of CLIO-NRP-V7 in detail (1). Initially, monocrystalline iron oxide (MION) was synthesized by neutralization of ferrous salts, ferric salts, and dextran with ammonium hydroxide, followed by ultrafiltration (10). The obtained MION (~2,064 iron atoms per nanoparticle) was cross-linked in strong base with epichlorohydrin and then reacted with ammonia to produce aminated iron oxide nanoparticle CLIO-NH_2. Next, fluorescein isothiocyanate (FITC)-labeled avidin was converted to its oxidized form using sodium periodate (1). The oxidized avidin was incubated with CLIO-NH_2 (pH 9.5) for 2.5 h to form Schiff base on the surface of CLIO, which was further reduced to a stable covalent bond by reacting with sodium cyanoborohydride for 3.5 h. The avidin molecule per CLIO nanoparticle ratio, which was ~2 avidin molecules per CLIO, was obtained by measuring the concentration of avidin and iron. The average number of biotin binding sites on avidin-CLIO was determined to be ~8 (4 for each avidin). NRP-V7 peptide was obtained with standard solid-phase

methods using fluorenylmethoxycarbonyl chemistry in an automated peptide synthesizer (2). H-2K^d molecules were immunoaffinity-purified from 1.4×10^{10} interferon (IFN)-γ–treated NIT-1 pancreatic β cells by using monoclonal antibody SF1-1.1(2). NRP-V7 formed stable complexes with H-2K^d molecule in aqueous solution. NRP-V7/H-2K^d was biotinylated with an enzymatic process (11). Then, CLIO-avidin was coupled with biotinylated monomer NRP-V7/H-2K^d at a molar ratio of 4 mol biotin per mol avidin to yield CLIO-NPR-V7 (1). The mean hydrodynamic sizes of the nanoparticles as measured with laser light scattering were 29.5 nm for CLIO-NH_2, 66.8 nm for CLIO-avidin, and 114.1 nm for CLIO-NRP-V7. CLIO-NRP-V7 was found to bind with high molecular weight biotinylated proteins such as albumin by measuring it spin-spin relaxation time (T_2) at 0.47 T.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Moore et al. examined the specificity of CLIO-NRP-V7 for NRP-V7–reactive CD8$^+$ TCs *in vitro* with the use of flow cytometry (1). CD8$^+$ TCs derived from 8.3-NOD mice or wild-type NOD mice were incubated with iodinated CLIO-NRP-V7 (50 μg Fe) for 1 hr, and then the TCs were analyzed with fluorescence-activated cell sorting (FACS) and quantified with γ-counting. The labeling efficiency of CLIO-NRP-V7 was found to be 92% in 8.3-NOD–derived CD8$^+$ TCs *versus* 1.86% in wild-type NOD mice, demonstrating a high specificity of CLIO-NRP-V7 for NRP-V7–reactive CD8$^+$ TCs. As found with fluorescence microscopy, CLIO-NRP-V7 was predominantly located inside the cell labeled by the internalization of TCR/CLIO-NRP-V7 complexes. CLIO-NRP-V7 appeared not to cause apparent cytotoxicity to the labeled cells, which demonstrated similar levels of cellular differentiation or secretion of cytokines such as interleukin-2 and IFN-γ. As a control, no uptake was found in NRP-V7–reactive CD8$^+$ TCs that were incubated with CLIO-NH_2 or CLIO conjugated with irrelevant peptides.

Medarova et al. examined the distribution of CLIO-NRP-V7–labeled CD8$^+$ TCs in the pancreata of NOD mice with *in vitro* immunofluorescence microscopy and histological analysis (5). The excised pancreata were stained with an anti-CD3 monoclonal antibody and hematoxylin & eosin (H&E) staining. CLIO-NRP-V7–labeled CD8$^+$ TCs were found in pancreatic islets and pancreatic lymph nodes as early as 5 weeks of age and reached a maximum at 8 weeks of age. The majority of CLIO-NRP-V7 was found to be localized in TCs. Light microscopy was also used to examine the excised pancreas stained with anti-CD8 (for CD8$^+$ TCs) or anti-CD68 antibody (for macrophages). CLIO-NRP-V7 was found in both macrophages and CD8$^+$ TCs. In comparison, NOD mice injected with CLIO-NH_2 exhibited limited accumulation of the probe only in macrophages.

Animal Studies

Rodents

[PubMed]

Moore et al. studied the *in vivo* binding of CLIO-NRP-V7 to autoreactive $CD8^+$ TCs (1). 8.3-NOD mice or wild-type NOD mice were injected with CLIO-NRP-V7 (10 mg Fe/kg) intravenously, and blood was withdrawn 24 h later for FACS analysis. The labeling efficiency was ~90% for the peripheral blood $CD8^+$ TCs derived from 8.3-NOD mice but ~2% for those derived from wild-type NOD mice. This result demonstrated that the systematic injection of CLIO-NRP-V7 allowed for selective *in vivo* labeling of NRP-V7–reactive $CD8^+$ TCs. Moore et al. further tracked the homing of the labeled NRP-V7–reactive $CD8^+$ TCs with *in vivo* MRI (1). NRP-V7–reactive $CD8^+$ TCs derived from 8.3-NOD mice were labeled *ex vivo* with CLIO-NRP-V7 followed by intraperitoneal transferr into NOD SCID mice (5 weeks old, $n = 5$). MRI images were collected before and 1, 2, 4, 5, 9, and 13 days after cell transfer. The pancreata became darker over time in T_2-weighted imaging, demonstrating the progressive accumulation of cells; no change in signal intensity was found in the spleen, liver, or muscle tissue. The change in signal intensity was noticeable from the second day after the cell transfer and continued up to day 16. The signal was diluted by *in situ* cellular proliferation of accumulated cells, but progressive recruitment and accumulation of labeled cells within the inflamed tissue compensated for this dilution. During this time, no alteration in blood glucose sugar was observed. To confirm the results, the animals were euthanized after MRI experiments, and pancreata were excised for histological staining and fluorescence microscopy analysis. H&E staining confirmed the infiltration of transferred lymphocytes in the islets. The fluorescence microscopy demonstrated the presence of FITC-positive $CD8^+$ TCs in the pancreas.

Medarova et al. used CLIO-NRP-V7 to study the infiltration of $CD8^+$ TCs in the pancreatic islets of NOD mice at different ages (5). CLIO-NRP-V7 (10 mg Fe/kg) was injected intravenously into female NOD mice at 5 ($n = 7$), 8 ($n = 4$), 15 ($n = 4$), and 24 ($n = 10$) weeks of age. T_2-Weighted images were collected before and 24 h after injection with a 9.4-T imager. As a control, the same dose of CLIO-NH_2 was injected to evaluate the contributions from nonspecific uptake of nanoparticles by macrophages. There was a significant change in pancreas for CLIO-NRP-V7 compared with that for CLIO-NH_2, demonstrating antigen-specific accumulation of CLIO-NRP-V7. The signal decrease in pancreas reached its maximum in 8-week-old NOD mice. The animals were euthanized after the MRI experiments; their blood, splenocytes, and islets were isolated for flow cytometry analysis. The percentage of MION-NRP-V7–reactive $CD8^+$ TCs in the blood peaked in 7- to 8-week-old mice but not in the spleen, indicating a high CLIO-NRP-V7–specific $CD8^+$ TCs in the intra-islet lymphocyte population in the circulating pool. This result demonstrated a good correlation between the flow cytometry analysis and the MRI finding that the CD8+ intra-islet lymphocytes were highest in 8-week-old NOD mice.

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.

NIH Support

DK 64850, DK 63572

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