TLR4 Peptide Inhibitor Set: VIPER

Catalog No:	NBP2-26244
Contents:	NBP2-26244A: VIPER, A TLR4 Inhibitory Peptide: 1 mg (lyophilized) KYSFKLILAEYRRRRRRRRR (VIPER sequence is underlined). Molecular weight: 2780.3 NBP2-26244B: CP7, Control Peptide: 1 mg (lyophilized) RNTISGNIYSARRRRRRRRR (Control sequence is underlined). Molecular weight: 2601
Storage:	The lyophilized peptides are stable in the dessicator at room temperature or at -20°C for at least 1 year.
Species Reactivity:	Human, Mouse
Form:	White Solid
Purity:	>95% by HPLC

Inhibitor Mechanism

VIPER binds to TLR4 and its adaptor proteins Mal and TRAM, interfering with TLR4/Mal and TLR4/TRAM interactions. Specifically, it is thought that the TLR4 inhibitor binds to the TIR domains of the receptor and adaptor proteins, thereby inhibiting TLR4 signaling by interfering with TLR4-Mal and TLR4-TRAM TIR-TIR interactions. However, the exact binding sites and inhibitory mechanism remain to be fully elucidated.

Background

VIPER (viral inhibitory peptide of TLR4) is a TLR4 specific inhibitory peptide that potently inhibits TLR4-mediated responses and is inert towards other TLR pathways (Bowie et al, 2010; Lysakova-Devine et al, 2010). The TLR4 inhibitor consists of an 11 amino acid (KYSFKLILAEY) inhibitory sequence, derived from the A46 vaccinia virus protein, linked to a 9R (RRRRRRRRR) homopolymer delivery sequence.

CP7 is an inert control peptide. The control consists of an 11 amino acid (RNTISGNIYSA) inert sequence also linked to a 9R homopolymer delivery sequence. The 9R sequence efficiently delivers the peptides into cells. CP7 is designed to be a negative control peptide for inhibitory assays.

We note that this inhibitor is the first viral inhibitory peptide identified that is specific for a particular TLR pathway. Inhibition of TLR4 within the context of the overall TLR signaling network is illustrated in Figure 1. VIPER should be useful for elucidating new mechanisms and molecular interactions involved in TLR4 signaling, and may also have potential for leading to the development of a new class of novel therapeutics that perturb TLR4 signaling by targeting Mal and TRAM TIR domains. The identification of additional short virally derived peptides that perturb TLR signaling is sure to open a new era in knowledge about how viruses specifically interact with host immune systems.

Solubility

Solubilize the peptides prior to use by preparing 5 mM stock solutions in sterile H20. The stock solutions are stable at -20oC for at least 2 months; avoid repeated freeze/thaw cycles and store in a non frost free freezer. The stock solutions may also be stored at 4 oC for up to 2 weeks.

Preparation

Preparation of 5 mM VIPER and CP7 Stock Solutions

Note: Bring the peptides to room temperature and quick spin the tubes before opening the caps.

VIPER: A final volume of 72 µl will make a 5 mM stock solution. Add 72 µl sterile H20 to the tube of peptide. Carefully pipet to ensure all of the peptide is dissolved.

CP7: A final volume of 76 µl will make a 5 mM stock solution. Add 76 µl sterile H20 to the tube of peptide. Carefully pipet to ensure all of the peptide is dissolved.

The stock solutions may be diluted further to make working solutions. Dilute according to the needs for your assay. For example dilute 5 mM stock solutions 1:10 in sterile 1X PBS or cell culture media to make 500 µM working solutions. Working solutions should be made fresh daily and not stored.

Usage

The inhibitor is used in assays to inhibit TLR4 activation; see Figure 1 and also refer to Lysakova-Devine et al (2010) for examples. Optimal inhibitor concentrations should be established through titration and may vary between model systems. We recommend an initial titration from 0-30 uM for in vitro assays (Fig 2). Control concentrations should mirror inhibitor concentrations. Inhibitor and control should be preincubated with cells prior to ligand activation to allow sufficient time for the peptides to enter from the media into the cell. We typically pre-incubate with inhibitor and control peptides for 2 h prior to TLR4 activation with LPS (Fig 2); however, optimal preincubation times may vary between model systems.

The TLR4/MD-2/CD14 stably transfected cell line (NBP2-26503) is a useful positive control model system for studying inhibition of TLR4 activation by VIPER (Fig 2). SEAP is used as a readout assay in Figure 2 to measure TLR4 inhibition.

A novel model system is shown in Figure 3 where TLR4 inhibitor peptide, but not CP7, inhibited TLR4 activation in Mal-deficient immortalized mouse bone marrow-derived macrophages (iBMDMs). In these iBMDMs, the inhibitor targets TLR4-TRAM, but not TLR-Mal, interactions as Mal is not expressed. TNF-alpha is used as a readout assay in Figure 3 to measure inhibition.

As applications and experimental paradigms vary, each researcher should determine the suitability of VIPER for their individual model systems. Likewise, each researcher should determine optimal concentrations, readout assays and other relevant parameters such as time courses for their model systems. Signal transduction pathways and model systems are complex, as such results may vary from examples shown herein or described in the published literature.

Note: VIPER has also been described for an in vivo model system where BALB/c mice were injected i.v. with 0.1 mg/kg of inhibitor peptide (Bowie et al, 2010; Lysakova-Devine et al, 2010).

Quality Control

TLR4 inhibitor and control peptides are quality controlled in vitro using the TLR4/MD-2/CD14/NF-kB/SEAP cell line (NBP2-26503) with SEAP (NBP2-25285) as a readout assay (Fig 2).

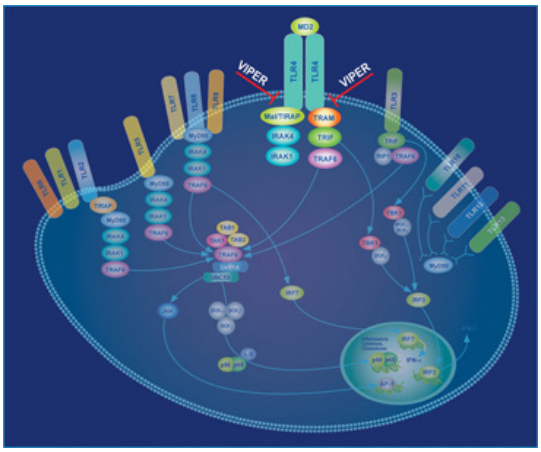

Figure 1: VIPER, TLR4 pathway specific inhibitor peptide. The inhibitor peptide inhibits TLR4 signaling by blocking interactions between TLR4 and its adaptors Mal/TIRAP and TRAM.

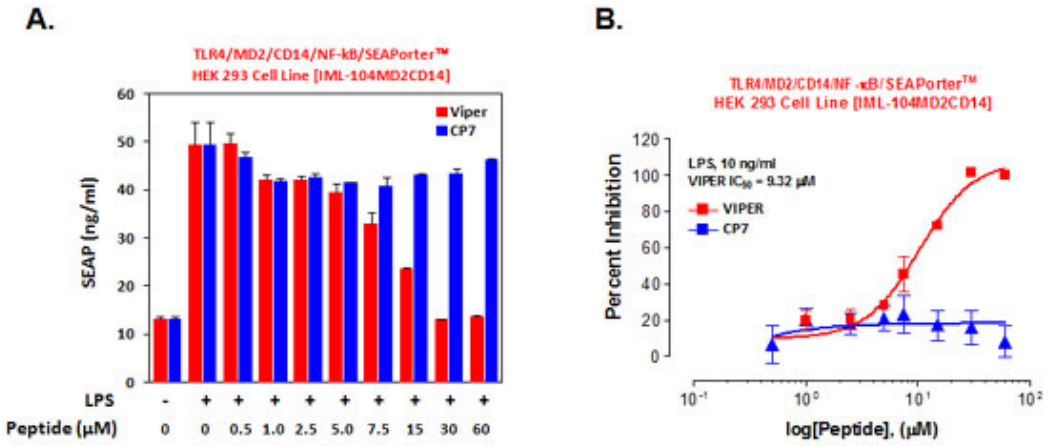

Figure 2. Evaluation of the TLR4 signaling inhibitor peptide. TLR4/MD-2/CD14/NF-kB/SEAPorter stably transfected cells (NBP2-26503) were plated in 96-well plates at 1x10 ^ 5 cells/well. After 16 h, cells were preincubated with various concentrations of inhibitor or control peptides (CP) for 1 h. Cells were then stimulated with 10 ng/ml LPS (NBP2-25295) for 24 h. The secreted alkaline phosphatase (SEAP) was analyzed using the SEAPorter Assay Kit (NBP2-25285). Inhibitory effect on the LPS-mediated TLR4 activation by the VIPER peptide is shown in panel A, and IC_{50} of the VIPER peptide is described in panel B.

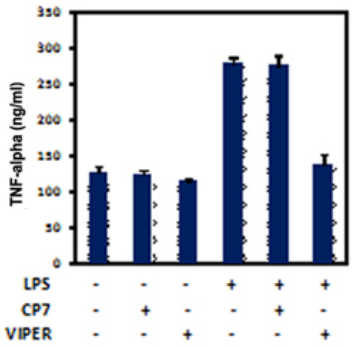

Figure 3: TLR4 inhibitor peptide inhibits LPS-induced activation in iBMDMs. iBMDM cultures were pretreated with 5uM of inhibitor or control prior to stimu-lation with 20 ng/ml LPS. Negative (LPS-, LPS-/CP7+, LPS-/CP7+) and positive (LPS+) controls were also included. Supernatants were harvested after 6 h and murine TNF-alpha measured by ELISA. TLR4 inhibitor, but not the control, inhib-ited LPS-induced TNF-alpha production. Image courtesy of Dr. Julianne Stack and Dr. Andrew Bowie, Trinity College, Dublin, Ireland.

Product Citations

1. Viral inhibitory peptide of TLR4, a peptide derived from vaccinia protein A46 specifically inhibits TLR4 by directly targeting MyD88 adaptor-like and TRIF-related adaptor molecule. Lysakova-Devine T, B Keogh, B Harrington, K Nagpal, A Halle, DT Golenbock, T Monie, AG Bowie. J Immunol 185:4261-4271 (2010). First VIPER publication, Table 1 and Figs 1-8. Figure highlights (see publication for additional details):

   Table 1: VIPER and CP7 peptide sequences

   Fig 1. Inhibition of LPS induced TLR4 activation in mouse RAW264.7 cells by VIPER but not the CP7 negative control. Specificity studies showing that VIPER is specific for TLR4 activation and does not inhibit Pam3CSK4 (TLR1/2), MALP2 (TLR6/2), CpG (TLR9) or Poly(I).Poly(C) (TLR3) activation . Readout assay: TNF-alpha ELISA.

   Fig 3: VIPER inhibition of LPS-induced activation in human THP-1 and PMBCs. Readout assay: TNF-alpha ELISA.

   Fig 4: VIPER inhibition of LPS-induced activation in an in vivo mouse model. Readout assay: IL-12/23 p40 secretion TLR4 Inhibitor Peptide (NBP2-26244)

Fig 5A, B, J, K. VIPER inhibition of LPS-induced activation in stimulated mouse bone marrow-derived macrophages. Readout assay: TNF-alpha and IFN-beta mRNA, Western blot assays. 2. A vaccinia virus protein A46 peptide and use thereof. Bowie AG, BN Harrington, TS Lysakova, B Keogh. International Patent Application WO 2010/055500 (05/20/2010). Filing date: 11/17/2009. http://www.wipo.int/patentscope/search/ VIPER inhibition of TLR4 activation is demonstrated in multiple figures, utilizing various assays, throughout the document.

3. MyD88 inhibition amplifies dendritic cell capacity to promote pancreatic carcinogenesis via Th2 cells. Ochi A, AH Nguyen, AS Bedrosian, HM Mushlin, S Zarbakhsh, R Barilla, CP Zambirinis, NC Fallon, A Rehman, Y Pylayeva-Gupta, S Badar, CH Hajdu, AB Frey, D Bar-Sagi and G Miller. J Exp Med 209(9): 1671-1687; doi:10.1084/jem.20111706 (2012). Novus products cited: TLR4 Peptide Inhibitor Set (NBP2-26244).

4. Poxoviral protein A46 antagonises Toll-like receptor 4 signaling by targeting BB loop motifs in Toll-IL-1 receptor adaptor proteins to disrupt receptor:adaptor interactions. Stack J, AG Bowie. JBC. http:www.jbc.org/cgi/doi/10.1074/jbcM112.349225 (2012).

   VIPER is derived from a surface patch of the 25 kDa vaccinia viral A46 protein, amino acids KYSFKLILAEY. The mechanism by which A46 disrupts normal TLR4 complex function remains to be fully elucidated. This publication shows that amino acids KYSFKLIL are essential for A46:TRAM interactions. Hence, this extends the knowledge of VIPER function indicating that these sequences in VIPER (KYSFKLIL) selectively target TRAM, thereby disrupting TLR:TRAM interactions. Refer to the publication and Figs 1-6, and Fig S1 for additional information.

5. Poxovirus A46 protein binds to TIR domain-containing Mal/TIRAP via an a-helical sub-domain. Oda S-i, E Franklin, AR Khan. Molecular Immunol 48:2144-2150 (2012). Biacore applications to determine binding of VIPER to Mal. Figs 4,5,6; Tables 1, 3. As expected, the results suggested that there is no significant affinity of VIPER for Mal in vitro.

6. Immunomodulatory effects of heat-killed Enterococcus faecalis TH10 on murine macrophage cells. Itoh T, Y Miyake, A Onda, J Kubo, M Ando, Y Tsukamasa, M Takahata. McrobiologyOpen 4:373-380 (2012). Readout assay: Nitric oxide (NO) production. VIPER but not the CP control peptide inhibited LPS induced nitric oxide (NO) production in mouse RAW264 cells (Fig 3A). However, VIPER did not inhibit Enterococcus faecalis TH10 (EF) bacterial induced NO induced production (Fig 3A), suggesting that this bacteria probably did not act through the TLR4 pathway.

   Note: Inhibition of LPS induced NO production by VIPER was used as a positive control for VIPER inhibition of the TLR4 pathway (Fig 3A).

7. Neuronal toll-like receptor 4 signaling induces brain endothelial activation and neutrophil transmigration in vitro. Leow0Dyke S, C Allen, A Denes, O Nilsson, S Maysami, AG Bowie, NJ rothwell, E Pinteaux. J Neuroinflammation 9:230 (2013), doi:10.1186/1742-2094-230. 1. Fig 2 (primary mouse neuron cultures). Readout assay: Chemokine CXCL1 (KC) ELISA. VIPER (but not the CP control peptide) when added at 2 um completely abolished LPS induced KC expression and release. Neither VIPER nor CP when used at 2 um had any effect on cell death or vability as assessed by LDH or MMT assays (data not shown). 2. Fig 5c (primary mouse endothelial cell cultures). Readout assay: neutrophil trans-endothelial migration. VIPER, but not CP, inhibited LPS induced infiltration of neutrophils across the endothelial monolayer.