Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Immunomodulatory Vasoactive Intestinal Peptide Amphiphile Micelles

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Supporting Figures

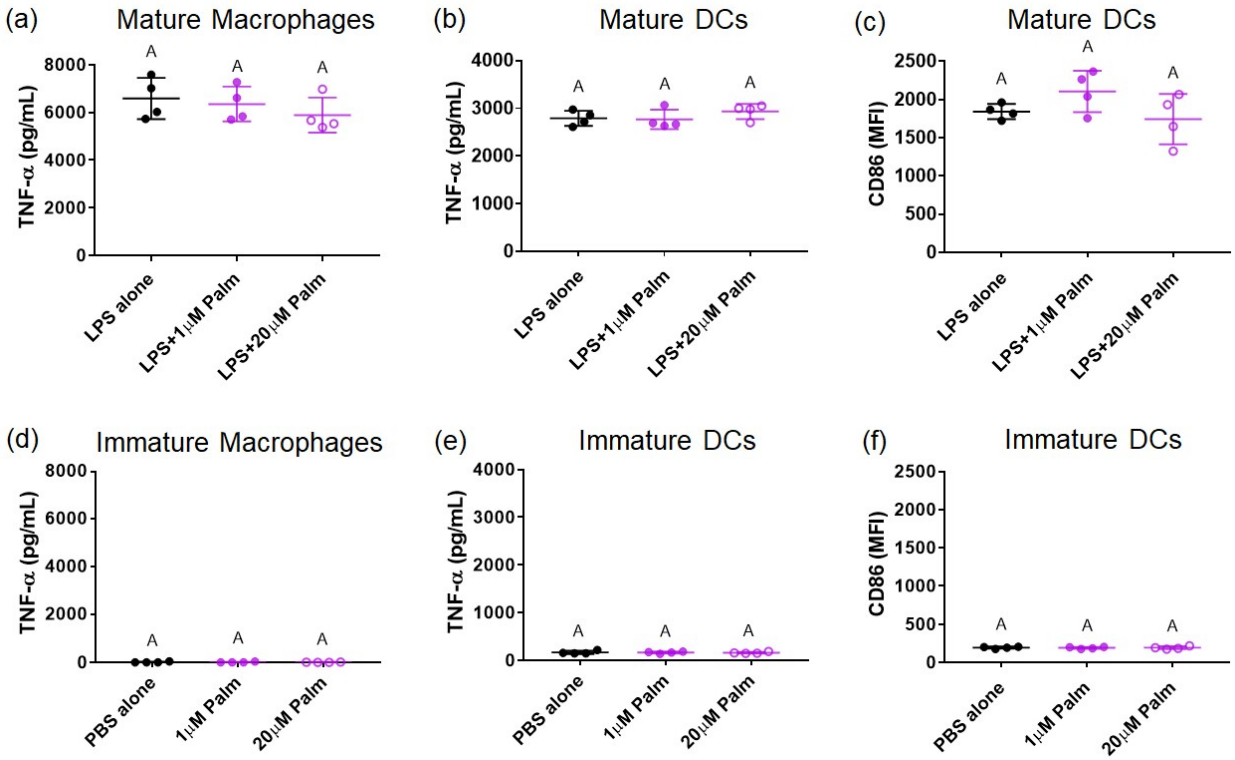

Figure S1. Inflammatory signal regulation of the lipid moiety (*i.e.* palmitic acid - Palm). TNF-α secretion from MØs or DCs, as well as CD86 expression from DCs was evaluated in the presence (a – c) or absence (d – f) of LPS. Palm was found to have no impact on regulating TNF-α secretion or CD86 expression for cells both at mature stages (a – c) or immature stages (d – f). Within a graph, groups that possess different letters have statistically significant differences in mean ($p \leq 0.05$) whereas those that possess the same letter are similar ($p > 0.05$).

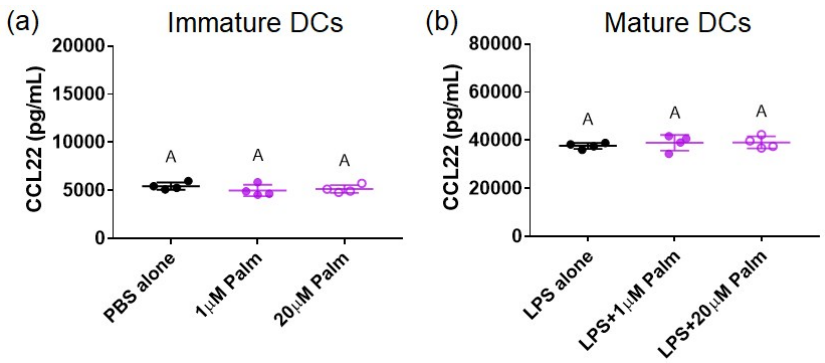

Figure S2. CCL22 induction effects of the lipid moiety (*i.e.* palmitic acid - Palm). The secretion of CCL22 from immature DCs (a) and mature DCs (b) was evaluated. Palm was found to have no impact on CCL22 induction regardless of DC maturation state. Within a graph, groups that possess different letters have statistically significant differences in mean ($p \leq 0.05$) whereas those that possess the same letter are similar ($p > 0.05$).

Materials and Methods

VIP and VIPA synthesis and purification: Side chain protected VIP and K-$(EK)_4$-VIP (zVIP) on rink amide resin was purchased from Synpeptide Co., Ltd (Shanghai, China). Palm tail modification was achieved by either direct conjugation to the peptide N-terminus (pVIPA) or amine side-group on the non-native N-terminal lysine (pzVIPA) in a glass reaction vessel (Chemglass, Vineland, NJ). VIP, pVIPA, and pzVIPA were cleaved from resin and their side groups deprotected via a single-step reaction consisting of a 2 hour exposure to the following mixture: trifluoroacetic acid (TFA), thioanisole, phenol, water, ethandithiol, and triisopropylsilane (87.5:2.5:2.5:2.5:2.5). Precipitation and multiple washings with diethyl ether yielded crude VIP, pVIPA, and pzVIPA. All products synthesized were characterized by analytical high-pressure liquid chromatography (HPLC, Beckmann Coulter, Fullerton, CA) and purified by mass spectrometry aided semi-preparative high-pressure liquid chromatography (LC-MS) using either a C4 or C18 column (Milford, MA) and in-house optimized solvent gradients. All reagents used were HPLC grade or peptide synthesis grade.

Critical micelle concentration (CMC): CMC was measured indirectly by 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence. DPH becomes significantly brighter when trapped within a hydrophobic domain so a rapid change in fluorescence corresponds to micelle formation. PA solutions were serially diluted in 1 μM DPH containing 0.01% THF and allowed to equilibrate for 1 h prior to fluorescence measurement (*ex*. 350 nm, *em*. 428 nm) by a BioTek Cytation 5 fluorospectrophotometer. The resulting data was fit with two trend lines from which the fluorescence inflection point was interpreted as the CMC.

Transmission electron microscopy (TEM): Micelle morphology was assessed by negative stain TEM with 5 μL of product solutions (100 μM) added to Glow-discharged, carbon support TEM grids (200 mesh, Electron Microscopy Sciences). After 5 minutes of incubation, filter paper was used to wick away excess solution and 5 μL of nanotungsten (Nanoprobes, Inc) was immediately added. After 5 minutes of incubation, grids were blotted dry and imaged with a FEI Tecnai F30 Twin TEM at 120 kV. At least 3 different spots on each grid were analyzed for which the images presented are representative ones. Tilt series images were collected at 200 kV, spot size 4, gun lens of 5, and extraction voltage of 3950 μA at a nominal 23,000x magnification with an underfocus of 1 μm. Tilt increments were collected every 2 degrees with a tilt range of +/- 70°, starting at 0°, with the negative half of the tilt series collected using FEI Xplore3D. Frames were aligned using IMOD with the patch-tracking algorithm using the entire imaged area for frame alignment and reconstructed with the weighted back projection algorithm.

Circular Dichroism (CD): Micelle secondary structure was investigated by circular dicroism using a circular dichroism spectrometer model 62DS (Aviv Biomedical, Inc, Lakewood, NJ). Micelle solutions (40 μM) were loaded into a 1 mm cuvette and measured a total of 10 times from 195 nm to 250 nm with an interval of 1 nm. The averaged data was curve fit using a linear combination of polylysine basis structures to calculate approximate α-helix, β-sheet, and random coil content.

Preparation of bone marrow derived dendritic cells (DCs): Balb/c mouse femurs and tibias were harvested from which cells were collected by flushing the bone marrow with complete RPMI 1640 media that was passed through a cell strainer (mesh size = 70μM). Red blood cells were lysed by ammonium-chloride-potassium (ACK) lysis buffer before stromal cells were seeded on non-tissue culture treated petri-dishes. The cells were cultured in DC differentiation media (RPMI 1640 supplemented with 10% fetal bovine serum - FBS, 1% penicillin-streptomycin, 50 μM β-mercaptoethanol, and 20 ng/mL granulocyte-macrophage colony-stimulating factor - GM-CSF) at 37 °C with 5% CO_2 for which culture media was refreshed on days 3, 6, and 8. Stromal cells were considered to have differentiated into DCs after 10 days of incubation.

In vitro VIP treatment and biological characterization: RAW 264.7 macrophages (MØs) and DCs were plated in 24-well tissue culture treated plates at 2×10^5 cells per well and incubated overnight. Cells were treated with different concentrations (1 μM or 20 μM) of one of the VIP products with or without the presence of 100 ng/mL lipopolysaccharide (LPS, Santa Cruz). After 24 hours, cell culture supernatants were collected and stored at - 80 °C until future analysis by a TNF-α enzyme-linked immunosorbent assay (ELISA, BioLegend) or a CCL22 ELISA (Thermo Fisher). DCs were harvested, blocked with anti-CD16/32 for 10 mins, and then stained with fluorescently-labeled antibodies (PE/Cy7-CD11c and PE-CD86, Biolegend) for 30 mins. Cells were fixed with 4% paraformaldehyde and analyzed via flow cytometry (BD LSRFortessa X-20).

Statistical analysis: JMP software (SAS Institute) was used to make comparisons between groups using an analysis of variance (ANOVA) followed by Tukey's HSD test to determine pairwise statistically significant differences ($p < 0.05$). Within graphs, groups that possess different letters have statistically significant differences in mean whereas those that possess the same letter are statistically insignificant.