Analogs of LDL Receptor Ligand Motifs in Dengue Envelope and Capsid Proteins as Potential Codes for Cell Entry

Short Title: LDL Receptor Ligand Motifs in Dengue Proteins

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SUPPLEMENTAL MATERIALS

Basic amino acid clusters in motifs, X**BBB**XX**B**X, X**BB**X**B**X, and Ψ**B**ΨX**B**, in apo E and apo B100 of the low-density lipoproteins, are known to bind the LDL receptors, heparin, and highly sulfated polysaccharides also occur in the polyproteins sequences of the Dengue viruses 1-4. The Ψ**B**ΨX**B** motif occurs in Domain III, the putative receptor binding region of the DENV envelope glycoproteins, twice in DENV 1 and 2, and once in DENV 3 and 4. The DENV2 synthetic peptide Dsp2EP-FITC that encompasses residues ^{0564}Gly-Gly^{0595} in the envelope protein region of the polyprotein and DENV3 Dsp3CP-FITC, ^{0002}Asn-Gln^{0028}, in the N-terminal capsid region of the DENV3 polyprotein were used to assess binding to Hep G2 cells and Liposorber Dextran Sulfate Cellulose Affinity beads of the Lipopheresis System.

Experiments with Hep G2 cells.

In humans, apo E is synthesized in several tissues primarily in the liver while apo B100 is synthesized in the liver and intestines [1, 2]. Hep G2 cells (human hepatocellular carcinoma cell line, HB-8065) obtained from the American Type Culture Collection (ATCC) Organization were used in binding experiments using Dsp2EP-FITC and Dsp3CP-FITC and binding experiments were performed as reported in the Methods section of the manuscript. Briefly, Hep G2 cells were propagated and maintained in 12 well culture trays containing 1 ml DMEM plus 10% Fetal Bovine Serum until approximately 50% confluence. Cells were then pre-conditioned to enhance expression of LDL receptors by removing the maintenance medium, rinsing the cells thrice with PBS, and next incubating the cells in 1 ml DMEM minus FBS for at least 2 hours at 37^{o} C in a 5% CO_2 atmosphere. Next, 10 µL of a solution containing 1 mg/mL Dsp2EP-FITC in PBS was added to the medium and cells then incubated as above for periods of 0, 30, 60, and 120 minutes, after which the binding assay mix was removed. Cells were then rinsed as before and fixed in PBS containing 1% formalin for 10 minutes. The fixing solution was removed, cells were rinsed as before with PBS and then maintained in 1 ml PBS for image acquisition. Results shown in Figure 1 (Frames C and D) indicate that Dsp2EP-FITC binds to Hep G2 cells. Frame B shows the typical clumping pattern seen with the Hep G2 cell type.

Experiments were also conducted to asses binding of peptide Dsp3CP-FITC to Hep G2 cells. Binding of this peptide to this cell type was not observed.

Hep G2 cells tend to form clumps in our cultures, and therefore, we considered this line unsuitable for our studies. Regardless, our observations suggest the DENV may have the capacity to enter hepatocytes through binding of the envelope glycoprotein. Our results indicate that this capacity is imparted by the Lysine/Arginine-based LDL receptor ligands motifs located between residues ^{0564}Gly-Gly^{0595}. It is interesting to speculate that Dsp3CP-FITC fails to bind to Hep G2 cells because the LDL receptors are occupied by apo E. A large fraction of apo E synthesized by hepatocytes is retained on the surface of the cell [3] and only a small fraction of what is internalized via endosomes is actually digested by liver cells, most is recycled out [4, 5].

Experiments with Dextran Sulfate Cellulose Resin.

The heparin and GAG binding properties of the low density lipoprotein particles, LDL, IDL, and VLDL, are well established. In these properties have been exploited in a clinical application as a rapid therapeutic method for lowering total cholesterol using dextran sulfate cellulose affinity chromatography. This procedure has been in practice for decades and is widely known as lipopheresis, also known as LDL apheresis. The Liposorber LA-15 Lipopheresis System uses dextran sulfate cellulose beads to lower LDL cholesterol levels in individuals unresponsive to lipid lowering medicaments. This procedure is apparently highly specific in removing LDL, IDL and VLDL directly from plasma because of its interaction with apolipoproteins B100 and E.

Liposorber beads obtained from Kaneka Pharma Corporation of Japan were recovered from the column and used to test binding of Dsp2EP-FITC. Beads were next suspended in PBS-5 mM $MgCl_2$ and 1.0 ml aliquots were added to vials, to each of which was added 10 µg of synthetic peptides, Dsp2EP-FITC, and mutated peptides Dsp2EP (B)-FITC and (C)-FITC. The mixtures were allowed to stand at ambient for 20 minute, centrifuged to pellet beads and washed thrice with buffer to remove unbound peptide. Results are shown in Figure 2.

Based on these results we conclude that DENV uptake may occur via the mechanisms used by lipoproteins, i.e. the LDL receptor, a GAG perhaps the heparan sulfate proteoglycans, or a combination of both HSPG and an LDL receptor or LRPs [6].

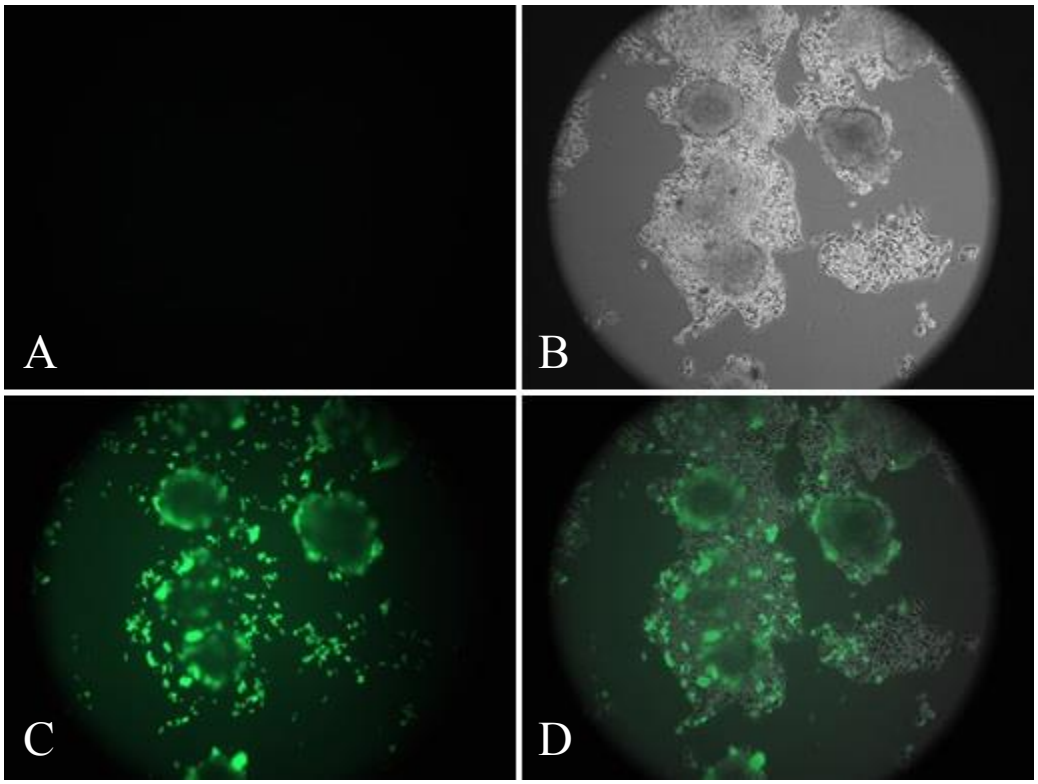

Figure 1 (Supplemental). Hep G2 cell binding of Dsp2EP-FITC. Image in Frame A shows the control assay which did not contain the Dsp2EP-FITC peptide. Images were captured using an Optronics Microfire CCD camera. Frame B shows bright light image of Hep G2 cells after incubating in binding assay for 60 minutes. Image in Frame C shows binding of Dsp2EP-FITC taken using the GFP filter and the Zeiss Axiovert microscope as described in Methods. Frame D shows an overlay image of B and C obtained using Photoshop CS4 software.

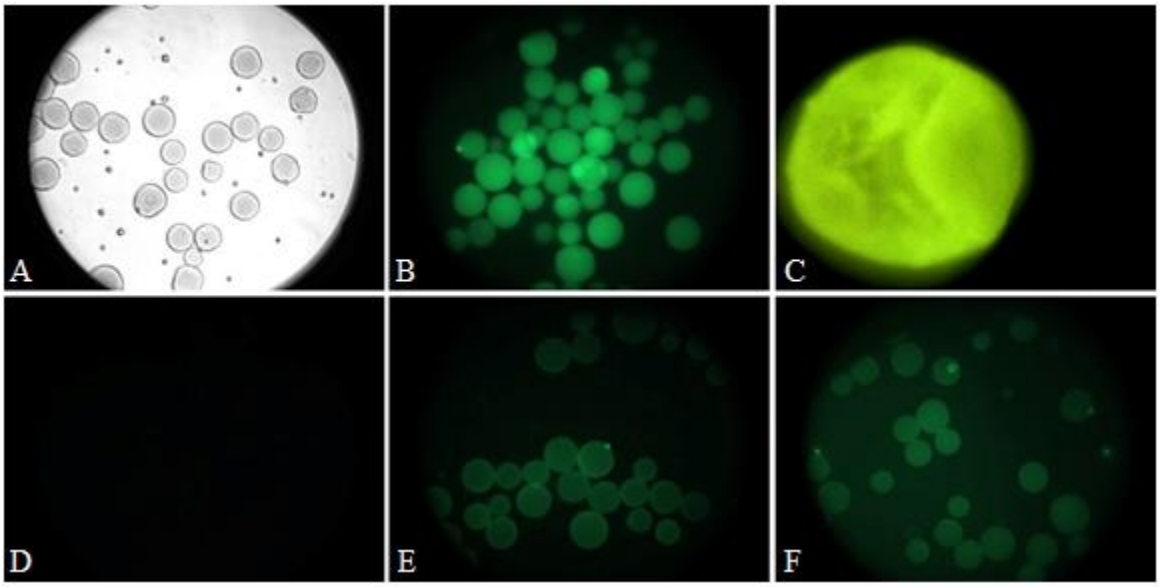

Figure 2 (Supplemental). Liposorber Dextran Sulfate Cellulose Beads binding to Dsp2EP-FITC and mutated synthetic peptides Dsp2EP (B)-FITC and Dsp2EP (C)-FITC. A bright light, gray scale image of the Liposorber beads is shown in Frame A. Image in Frame B shows that Dsp2EP-FITC binds these beads with apparent high avidity. Frame C image shows a highly magnified bead in which individual clusters of LDL particles are almost discernible. Frame D is a control image of beads to which no peptide was added. Frames E and F show low level binding of mutated peptides Dsp2EP (B)-FITC and (C)-FITC, respectively.

References

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