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Supplemental Information

DC-SIGN as a Receptor

for Phleboviruses (*Bunyaviridae*)

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cells, Antibodies, plasmids, siRNAs, and reagents

All cell lines were cultured according to ATCC recommendations. The neutralizing anti-DC-SIGN mouse mAb IgG2a (mAb 1621) and the anti-DC-SIGN phycoerythrin (PE)-conjugated fragment antigen binding (Fab) 1621P were purchased from R&D Systems. The mAb DC28 is a mouse Ab raised against the DC-SIGN ectodomain (Santa Cruz Biotechnology). As controls, mouse isotype IgG2a was used. Rabbit polyclonal Abs (pAbs) against the UUKV glycoproteins recognized linear epitopes in G_N and G_C. The mouse mAb 8B11A3 is directed against a linear epitope in the N protein (Persson and Pettersson, 1991). These three Abs against UUKV proteins are kind gifts from A.K. Överby and Ludwig Institute for Cancer Research (Stockholm branch). Anti-GFP rabbit pAbs were purchased from Acris Antibodies. Rabbit pAbs against the RVFV nucleoprotein N and all UUKV proteins (U2) were made in the house. Briefly, antisera against purified UUKV were prepared in rabbits by injecting 200 µg of virus proteins in Freund complete adjuvant. Two booster injections of 200 µg each in Freund incomplete adjuvant were injected at 4-week intervals. Rabbits were bled before immunization (control preimmune serum) and 2 weeks after the last injection. The plasmids pTripΔU3-DC-SIGN and -DC-SIGN LL (kind gifts from A. Amara) encode human DC-SIGN and its endocytosis-defective mutant respectively (Lozach et al., 2005). The plasmid encoding for EGFP-LAMP-1 has been previously described (Lozach et al., 2010). The pHR-SEW-mEGFP-DC-SIGN encodes mEGFP-DC-SIGN, which refers to DC-SIGN tagged by monomeric EGFP (Zacharias et al., 2002). UUKV siRNA (siUUKV_N, GGAGAUCUUGGAUGCCAAUGA) and AllStars negative-control siRNA (siCtrl) were from Qiagen. siUUKV_N was directed against the positive strand of the UUKV RNA segment coding for the nucleoprotein N. NH_4Cl and mannan stocks were from Sigma and dissolved in water. Bafilomycin A1 (Sigma) was dissolved in absolute methanol.

Construction of mEGFP-DC-SIGN

The DC-SIGN sequence was amplified by polymerase chain reaction (PCR) (DyNAzyme, Finnzymes) from the plasmid pTripΔU3-DC-SIGN using the sense primer 5'-AATATTGGATCCATGAGTGACTCCAAGGAACC-3' and the antisense primer 5'-AATATTGTCGACCTACGCAGGAGGGGGG-3'. The PCR product was digested with BamHI and SalI and introduced in the BglII-SalI-digested pmEGFP-C1 vector to generate the mEGFP-DC-SIGN sequence. pmEGFP-C1 was obtained by swapping of EGFP against mEGFP in pEGFP-C1 (Clontech) (a kind gift from A. Hayer). The mEGFP-DC-SIGN sequence was amplified by PCR using the sense primer 5'-AATATTGGATCCCGCCACCATGGTGAGCAAGGGCG-3' and the antisense primer 5'-AATATTGTCGACCTACGCAGGAGGGGGG-3' and then digested with BamHI and SalI. The digested product was introduced in BamHI-XhoI-digested pHR-SEW lentiviral vector (a kind gift from M. Grez).

DNA transfection

HeLa-DC-SIGN cells in 8w-chamber Lab-Tek II (Nalge Nunc) were transfected with plasmid encoding EGFP-LAMP-1 using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Protein analysis

Proteins were analyzed by SDS-PAGE and transferred to PVDF membranes (iBlot Transfer Stacks, Invitrogen). Incubation with primary Abs against UUKV glycoproteins (1:2000), GFP (1:2000), and DC-SIGN (DC28, 1:2000) was followed by incubation with anti-mouse or anti-rabbit horseradish peroxidase-conjugated secondary Abs.

FACS assay

As previously described (Lozach et al., 2010), infected cells were fixed and then permeabilized with a saponin-based buffer to stain intracellular viral antigens with antibodies. Briefly, permeabilized cells were incubated with primary Ab for 1 h, washed, and subsequently exposed to an anti-mouse or an anti-rabbit AF647-conjugated secondary Ab (1:500, Molecular Probes) for 45 min. Anti-virus-specific hyper immune ascites fluids (HMAFs) (1:200) were utilized to detect PTV and TOSV infection. A rabbit pAbs (1:200) against the nucleoprotein N was used to detect RVFV infection. The anti-N mouse mAb 8B11A3 (1:400) was used to detect cells infected with UUKV. When mAbs were used to neutralize or detect DC-SIGN in infection assays, UUKV-infected cells were stained with the anti-UUKV rabbit pAbs U2 (1:400). The infection index was determined by FACS analysis with a FACS Calibur cytometer (Becton Dickinson) and using Flowjo software (Treestar).

Immunofluorescence staining

The expression of DC-SIGN and mEGFP-DC-SIGN was assessed at the surface of cell (not permeabilized) by FACS using the anti-DC-SIGN PE-conjugated Fab 1621P (1:50) according to a standard procedure (Lozach et al., 2005). Surface expression of mEGFP-DC-SIGN was also detected with confocal microscope using the primary mAb DC28 (1:1000) and secondary anti-mouse AF594-conjugated Abs (1:500, Invitrogen). Total expression of DC-SIGN was assessed using confocal microscope or in infected cells using FACS. Briefly, after fixation and permeabilization with a saponin-based buffer, cells were incubated with the PE-conjugated Fab 1621P (1:50) or, alternatively, with the primary mAb DC28 (1:1000) and anti-mouse AF488-conjugated secondary Abs (1:500, Invitrogen).

SUPPLEMENTAL REFERENCES

Persson, R., and Pettersson, R.F. (1991). Formation and intracellular transport of a heterodimeric viral spike protein complex. J Cell Biol *112*, 257-266.

Zacharias, D.A., Violin, J.D., Newton, A.C., and Tsien, R.Y. (2002). Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science *296*, 913-916.