

Env ERVWE1 interaction with DC-SIGN.

Binding of the SU-N, SU-C and SU proteins to oligomeric DC-SIGN was tested by using a cell-ELISA approach described in [Erdile LF et al., 2001 ; Kataoka HN et al., 2000] and adapted to DC-SIGN. Arrow indicates the inability to detect the binding of SU-Cprotein to DC-SIGN molecules due to the absence of ant-SU-EnvW epitope onto SU-C protein. M eans +/- standard deviation ; n=5.

Stable DC-SIGN-expressing HeLa cells or parental HeLa cells were seeded in 96-well plates at 4 x 104 cells per well. The cells were left to adhere overnight, rinsed once and fixed by 4% paraformaldehyde in adhesion buffer (20 mM TRIS HCl pH8, 450 mM NaCl, 2 mM MgCl2 and 1 mM CaCl2, adapted from [Geijtenbeek TB et al., 2000]) for at least 15 min. After saturation, supernatants were added and incubated for 1h30. Cells were saturated for one hour with 10% nonfat dry milk in adhesion buffer and rinsed three time s with wash buffer (adhesion buffer containing 0.1% Tween 20). The detection was carried out using an anti-His-Mab at dilution of 1:1,000 in adhesion buffer supplemented with 1% BSA. Following 1 hour of incubation at 37°C, the plates were rinsed three times with wash buffer, and 1 µg/ml of horseradish peroxydaseconjugated antibody was added to each well and incubated at 37°C for 1 hour. The plates were washed three times with wash buffer and 100 µl of a mixture of H2O2 and o-phenylenediamine (Sigma Aldrich) was added for colorimetric detection. The plates were left for 10 min at room temperature in the dark and reaction was then stopped by adding 100 µl of 1M H2SO4. Absorbance was measured at 492 nm. To take into account the non-specific binding, assays were both carried out on stable DC-SIGN expressing HeLa cells and on parental HeLa cells.

Erdile, L. F., D. Smith, and D. Berd. 2001. Whole cell ELISA for detection of tumor antigen expression in tumor samples. J Immunol Methods 258:47-53.

Kataoka, H., N. Kume, S. Miyamoto, M. Minami, T. Murase, T. Sawamura, T. Masaki, N. Hashimoto, and T. Kita. 2000. Biosynthesis and post-translational processing of lectin-like oxidized low density lipoprotein receptor-1 (LOX-1). N-linked glycosylation affects cell-surface expression and ligand binding. J Biol Chem 275:6573-9)
Geijtenbeek, T. B., R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, G. J. Adema, Y. van Kooyk, and C. G. Figdor. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 100:575-85)



ERVWE1 is associated with detergent-resistant membranes (DRMs):

transfected with wild-type HERV-W plasmid or with amphotropic MLV envelope expressor plasmid (phCMV-A). 36 hours later, cells were washed in PBS and lysed in 1ml 1%Triton X-100 TNE buffer (150mM NaCl; 25mM Tris pH7.5 ; 1mM EDTA containing Complete-Mini protease inhibitor cocktail, Roche) 30 min on ice. Subsequently, lysates were mixed with 2ml of 45% sucrose (wt/vol in TNE) and placed in SW41 centrifuge tube (Beckman). Samples were overlaid with 6ml of 30% sucrose followed by 2.5ml 5% sucrose and spun 22 hours 38,000 rpm at 4°C. The gradient was collected in 1ml steps from the top to the bottom (1 to 12) and the pellet was collected in 1ml TNE and sonicated (P). Aliquots of 30µl of each fraction were analyzed by 9% polyacrylamide gel under non-reducing conditions. Blots were probed with anti-TM-Mab for Env ERVWE1 at dilution of 1:5,000 and with anti-SU-MLV for EnvA-MLV (Quality Biotech) at dilution of 1:1,000. The bulk of detergent-insoluble material is concentrated in fractions 3 and 4, whereas soluble material ends up in fractions 10 to 12.