Retrovirology



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Development of a CCR5-tropic HIV-I fusion inhibition assay amenable to high-throughput screening for topical microbicides

B Beer*1, B Snyder1, K Luckenbaugh1, C Lackman-Smith1, P Hogan1, R Ptak1, N Shindo2, L Rasmussen2, EL White2, A Brelot3 and M Alizon3

Address: ¹Southern Research Institute, Frederick, Maryland, 21701, USA, ²Southern Research Institute, Birmingham, Alabama, 35205, USA and ³Institut Cochin, INSERM U567, Paris, France

* Corresponding author

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Background

The development of safe and effective topical microbicides to limit the continuing AIDS pandemic is a high priority. The current NIAID microbicide testing algorithm includes both CCR5 (R5)-tropic and CXCR4 (X4)-tropic attachment inhibition assays, and an X4-tropic fusion inhibition assay. To complete this testing algorithm, an R5-tropic fusion inhibition assay was developed. The rationale for this development was that mucosal HIV-1 infection is primarily mediated through the R5 receptor and an R5-tropic entry inhibitor should be included in combination microbicide prophylaxis.

Materials and methods

The assay employs ADA/ENV cells (an HIV-1 ADA envelope and Tat expressing HeLa cell line) as the effector cells and MAGI-R5 (HeLa cells expressing CD4, R5, and an HIV-1 LTR driven β -galactosidase reporter) as the target cells. Upon co-culture of the two cell lines, HIV-1 envelope mediated cell-to-cell fusion occurs resulting in transactivation of the β -galactosidase reporter gene.

Results and Conclusion

Varying effector-to-target cell ratios were evaluated and a ratio of 1:2 was found to be optimal for the assay (i.e., 5×103 effector and 104 target cells). The R5 inhibitors SCH-D, SCH-C, Maraviroc, and TAK-779 were highly active in the assay, with 50% inhibitory concentrations ranging from 1 to 40 nM. A proprietary fusion peptide, based on

the C34 peptide, was also active. As expected, the X4 inhibitor AMD3100, Dextran, and all tested NRTIs, NNRTIs, and protease inhibitors were inactive in the assay. In order to determine suitability for high throughput screening, the robustness of the assay was assessed by determining the Z'-value in a 96-well plate format, yielding a Z'-value of 0.7. Assay validation in the 384-well plate format is in progress. (This work was funded by contract N01-AI-05415; Dr. Roger Miller, Project Officer.)