Retrovirology

Poster presentation

P19-41. Full-length IMC expressing Renilla luciferase for neutralization assay using PBMC

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Background

Neutralizing antibody is a crucial parameter in the evaluation of vaccine candidates and therefore, generating a relevant neutralization assay has been a major focus of research. Four critical variables are the viruses, the cells they were grown in, the target cells, and the mode of detection used in the assay. In order to standardize the PBMC-based assay, we are generating primary isolate-derived full-length infectious molecular clones (IMC) from HIV+ individuals representing subtypes A, B, C, D, CRF01_AE, and CRF02_AG. Each of these primary isolate IMCs is then modified to express the Renilla luciferase gene.

Methods

Renilla Luciferase gene isolated from pNL4.3_LucR.T2A (C. Jambor) was inserted in full-length HIV-1 IMC. The cloning was done without deleting any part of the HIV genome. For each IMC_LucRT2A generated, the corresponding env was cloned in pDNA3.1His to be used in pseudovirus TZM-bl-assay. Viral stocks were generated by transfection of 293T cells. Every construct was fully sequenced; only sequences with 100% amino acid sequence homology with the parental IMC were retained.

Results

We have so far generated 3 IMC_LucR; B-pWR27-1_LucR.T2A (subtype B), C-pETH2220-11B_LucR.T2A (subtype C) and 01-pCM235-2_LucR.T2A (CRF_01A/E). The IMC_LucR.T2A were tested for infection in TZMbl cells and PBMC. Compared with cognate IMC, the IMC_LucR.T2A showed a similar profile of infection. TZMbl NAb assays with different concentrations of monoclonal antibodies (4E10, 2G12 and, b12), soluble CD4 (sCD4), Clade B and C serum pools showed an equivalent 50% neutralization titer when comparing IMC and corresponding IMC_LucRT2A with non-significant, <3 fold difference between IMC and IMC_LucRT2A.

Conclusion

These encouraging data suggest that the IMC-LucRT2A primary isolate viruses may be used as a substitute for the traditional p24-based PBMC NAb assay.