Abstract

Emergence of SIV and HIV specific CD8 T cells has been shown to correlate with control of in vivo replication. Poor correlation between IFN-γ ELISPOT responses and in vivo control of the virus has triggered the development of more relevant assays to assess functional HIV-1 specific CD8 T-cell responses for the evaluation and prioritization of new HIV-1 vaccine candidates. We previously established a viral inhibition assay (VIA) that measures the ability of vaccine-induced CD8 T-cell responses to inhibit viral replication in autologous CD4 T cells. In this assay, viral replication is determined by measuring p24 in the culture supernatant. Here we describe the development of a novel VIA, referred to as IMC LucR VIA that exploits replication-competent HIV-1 infectious molecular clones (IMCs) in which the complete proviral genome is strain-specific and which express the Renilla luciferase (LucR) gene to determine viral growth and inhibition. The introduction of the luciferase readout does provide significant improvement of the read out time. In addition to switching to the LucR read out, changes made to the overall protocol resulted in the miniaturization of the assay from a 48 to a 96-well plate format, which preserved sample and allowed for the introduction of replicates. The overall assay time was reduced from 13 to 8days. The assay has a high degree of specificity, and the previously observed non-specific background inhibition in cells from HIV-1 negative volunteers has been reduced dramatically. Importantly, we observed an increase in positive responses, indicating an improvement in sensitivity compared to the original VIA. Currently, only a limited number of "whole-genome" IMC-LucR viruses are available and our efforts will focus on expanding the panel to better evaluate anti-viral breadth. Overall, we believe the IMC LucR VIA provides a platform to assess functional CD8 T-cell responses in large-scale clinical trial testing, which will enhance the ability to select the most promising HIV-1 vaccine candidates capable of controlling HIV-1 replication in vivo