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PCR as a Virological Endpoint for Testing Microbicide Efficacy in the Colorectal Explant Model

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Background

The *ex vivo* mucosal explant model is used to test the efficacy of microbicides that have the potential for preventing HIV-1 transmission. In the colorectal model, biopsies are challenged *ex vivo* with HIV-1 after study participants are exposed to microbicides *in vivo*. Product efficacy is assessed by the extent of HIV-1 p24 suppression in supernatant fluids sampled up to Day 14 after HIV-1 challenge. The purpose of this study was to determine if measurement of HIV-1 nucleic acids by real-time PCR and HIV-1 integration by Alu-gag PCR could be used as alternative endpoints in explant studies.

Methods

Twenty rectal biopsies each, from five HIV-1-negative individuals, were placed in culture medium and challenged with 1 x 10^5 virions /mL of HIV-1_{BaL} for two hours at 37° C. Biopsies were then washed and returned to the tissue culture incubator. Supernatant fluids were collected on Days 1, 2, 4, 6, 11 and 14 after infection and viral RNA was extracted (QIAamp Viral RNA Mini kit, Qiagen, Valencia, CA). Individual biopsies were harvested at the same time points and viral nucleic acids were extracted using the AllPrep DNA/RNA mini kit (Qiagen, Valencia, CA). HIV-1 RNA levels were measured by two different real-time PCR assays and a laboratory-developed nested PCR protocol. HIV-1 provirus was measured by real-time PCR assays targeting different regions of the HIV-1 genome as well as nested PCR. Alu-gag PCR followed by real-time PCR of the HIV-1 gag region was used to assess viral integration.

Results

Real-time PCR assays detecting HIV-1 DNA and RNA performed similarly provided there were no mismatches between the sequences of the infecting virus and sequences of the assay primers and probes. Viral RNA in supernatant fluids and biopsies could be detected at an earlier time point (Day 4) than HIV-1 provirus (Day 6). A 15 cycle nested PCR step prior to real-time PCR increased assay sensitivity of both HIV-1 RNA and proviral assays while maintaining levels that fell within standard curves. HIV-1 integration using Alu-gag PCR was measurable by Day 11 and 14 after infection,

Conclusions

Real-time PCR is a sensitive and accurate end point for assessing levels of HIV-1 infection in the *ex vivo* mucosal explant model. RNA assays are more sensitive than DNA assays all of which coincide with the time course of p24 accumulation in supernatant fluids. Integrated virus is detectable at later time points than viral RNA and DNA in biopsies but is measurable by Day 6 after infection in individual biopsies. The use of molecular techniques to characterize explant HIV infection provides an alternative to HIV-1 p24 supernatant testing. It may reduce the explant incubation period, requires minimal volumes of explant supernatant, is sensitive and has a broad dynamic range. Further evaluation of these techniques in screening the efficacy of microbicide candidates is warranted.

References

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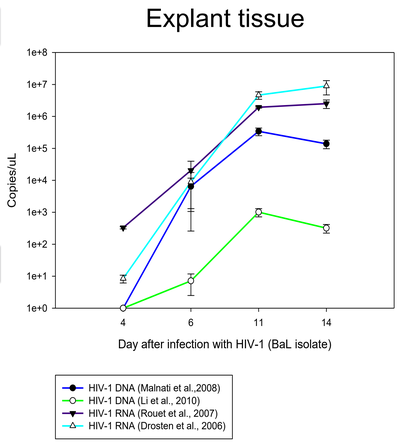


Figure 1. Detection of HIV-1 accumulation in mucosal biopsies after HIV-1 Infection. Twenty biopsies from the same individual were infected with 1 x 10^5 virions /mL of HIV-1_{BaL} and harvested at Days 4, 7, 11 and 14 after infection. HIV-1 RNA and DNA were extracted simultaneously from the same tissue and quantified by real-time PCR assay for HIV-1 RNA (Rouet et al. 2007; Drosten et al., 2006) and HIV-1 provirus (Malnati et al., 2008; Li et al., 2010). N=4 at Days 4, 7 and 11; N=8 at Day 14.

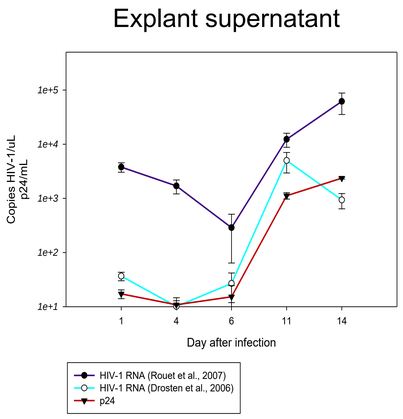


Figure 2: Accumulation of HIV-1 RNA in supernatant fluids following HIV-1 Infection. Biopsies were infected with 1 x 10^5 virions / mL HIV-1_{BaL}. Supernatant fluids were collected from the culture medium at Days 1, 4, 6, 11 and 14 after infection. HIV-1 RNA was quantified by real-time PCR and viral protein was measured by p24 ELISA. N=20, Days 1 and 4; N=16, Day 6; N=12, Day 11, N=8, Day 14.

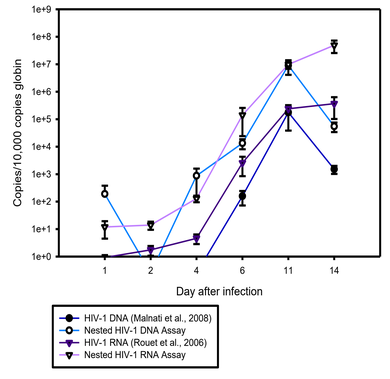


Figure 3. Accumulation of HIV-1 nucleic acids in mucosal biopsies: Comparison of real-time PCR with nested PCR. Twenty biopsies each, from 4 individuals, were infected in explant with 1 x 10^5 virions / mL HIV-1_{BaL} and harvested at Days 1, 2, 4, 6, 11 and 14 after infection. HIV-1 RNA (Rouet et al., 2007) and provirus (Malnati et al., 2008) were quantified by real-time PCR. HIV-1 was measured by nested PCR for each of the assays. The first step of each nested PCR reaction was carried out for 15 cycles prior to real-time PCR.

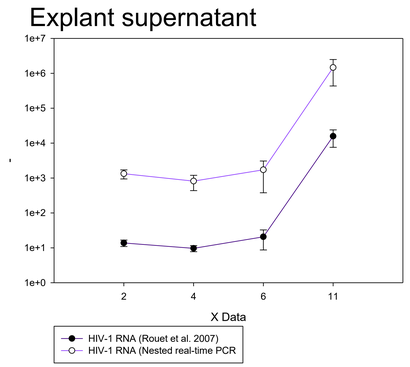


Figure 4. HIV-1 RNA Accumulation in supernatant fluids: Comparison of real-time PCR with nested PCR. Biopsies from each of 4 individuals were infected with 1 x 10^5 virions / mL HIV-1_{BaL} and supernatant fluids were collected at Days 1, 2, 4, 7, 11 and 14 after infection. RNA was extracted and quantified by real-time PCR and nested PCR. The first step of the nested PCR protocol was carried out for 15 cycles prior to real-time PCR. N=20, Day 1; N=16, Day 2; N=12, Day 4; N=8, Day 6; N=4, Day 11.

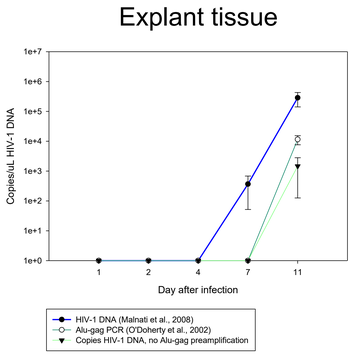


Figure 5. Detection of integrated HIV-1 DNA in biopsy tissue after infection with HIV-1_{BaL}. Biopsies were infected with 1 x 10^5 virions HIV-1_{BaL} and nucleic acids were isolated at Days 1, 4, 7 and 11 after infection. Alu-gag PCR was performed for 30 cycles (O'Doherty et al., 2002) followed by quantification of the PCR products by real-time PCR. Non-integrated DNA was measured on samples without prior Alu-gag PCR amplification and subtracted from total quantified Alu-gag PCR products to give integrated HIV-1 DNA.

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