Virology 499 (2016) 298–312

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Pathogenic infection of Rhesus macaques by an evolving SIV-HIV derived from CCR5-using envelope genes of acute HIV-1 infections

Mohammed Asmal [a], Sophie Lane [a], Meijuan Tian [b,c], Gabrielle Nickel [b], Colin Venner [c], Brennan Dirk [c], Jimmy Dikeakos [c], Corinne Luedemann [a], Linh Mach [a], Harikrishnan Balachandran [a], Adam Buzby [a], Srinivas Rao [a], Norman Letvin [a], Yong Gao [b,c], Eric J. Arts [b,c,*]

[a] Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA
[b] Division of Infectious Diseases and HIV Medicine, Case Western Reserve University, Cleveland, OH 44106, USA
[c] Department of Microbiology and Immunology, Schulich School of Medicine and Dentistry, Western University, London, ON N6A 5C1, Canada

ARTICLE INFO

Article history:
Received 20 July 2016
Returned to author for revisions
15 September 2016
Accepted 22 September 2016

Keywords:
SIV-HIV chimeric viruses
Envelope
Rhesus macaque
Pathogenesis

ABSTRACT

For studies on vaccines and therapies for HIV disease, SIV-HIV chimeric viruses harboring the HIV-1 *env* gene (SHIVenv) remain the best virus in non-human primate models. However, there are still very few SHIVenv viruses that can cause AIDS in non-CD8-depleted animals. In the present study, a recently created CCR5-using SHIVenv_B3 virus with *env* gene derived from acute/early HIV-1 infections (AHI) successfully established pathogenic infection in macaques. Through a series of investigations on the evolution, mutational profile, and phenotype of the virus and the resultant humoral immune response in infected rhesus macaques, we found that the E32K mutation in the Env C1 domain was associated with macaque pathogenesis, and that the electrostatic interactions in Env may favor E32K at the gp120 N terminus and "lock" the binding to heptad repeat 1 of gp41 in the trimer and produce a SHIVenv with increased fitness and pathogenesis during macaque infections.

1. Introduction

Nonhuman primate (NHP) trials play a key role in the iterative improvement of HIV-1 candidate vaccines (Staprans, Feinberg et al., 2010). In order to assess the efficacy of potential vaccines in NHPs, NHP challenge viruses are required that present the primary HIV-1 vaccine targets (i.e. HIV-1 Env) and also accurately reflect the sensitivity of transmitted HIV variants to vaccine-mediated immune responses. Chimeric viruses, which contain HIV Env, Rev, Vpu and Tat, with the remainder of the virus originating from the simian immunodeficiency virus (SIV), have been used as challenge viruses and to test the ability of antibodies to prevent infection (Barnett et al., 2010; Fouts et al., 2015; Barouch et al., 2013a, 2013b). Most HIV envelopes (especially CCR5-tropic Envs) do not produce viable viruses when introduced into the SIV backbone. Even though a recent study showed that mutations in *env* genes could significantly enhance their binding affinity to RhCD4 and viral replication in rhesus macaques (Li et al., 2016), the currently available SHIVs for use as challenge viruses in NHP have shortcomings. SHIVenv viruses with exclusively CCR5-tropic envelopes have been generated but upon infection of macaques, the loss of CD4 T cells is negligible or inconsistent and high viral loads are typically transient followed by eventual viral clearance. There are few cases of NHPs infected with R5 SHIVenv viruses (e.g. SHIV_SF162-P3, SHIV_Ba-L, SHIV_2873Nip, and SHIV_1157ipd3N4) that exhibit an early peak viremia, sustained high viral loads (10^6–10^8 copies/mL) with no reduction to a viral load set point and rapid progression to mortality, i.e. before an effective immune response is elicited (Tan et al., 1999; Pahar et al., 2007; Pal et al., 2003; Song et al., 2006; Humbert et al., 2008). Recently, pathogenic HIV infections were described in pigtailed macaques (*Macaca nemestrina*) depleted for CD8+ T cells and where HIV-1 harbored SIV_{MAC} Vif to reduce restriction by APOBEC3 proteins (Hatziioannou et al., 2014). In cases where CD8 T cells are not of importance, Hatziioannou et al. (2014) was a breakthrough for use of pigtail macaques in HIV vaccine, pathogenesis, and treatment studies. In a study by the same group and at the same time as this

Abbreviations: HIV-1, human immunodeficiency virus type 1; SHIV, simian/human immunodeficiency virus; AHI, acute HIV-1 infections

* Corresponding author at: Department of Microbiology and Immunology, Schulich School of Medicine and Dentistry, Western University, London, ON N6A 5C1, Canada.
E-mail address: earts@uwo.ca (E.J. Arts).

http://dx.doi.org/10.1016/j.virol.2016.09.021
0042-6822/

study, rhesus macaques depleted for CD8+ T cells were infected with pools of SHIVenv viruses (also derived from acute HIV-1 env genes) (Del Prete et al., 2014). The most recent study showed that, even with the Envs that could bind to RhCD4 with high affinity, the infection of rhesus macaques only resulted in AIDS in less than 10% (1 out 12) of non-CD8-depleted macaques, compared to 50% of CD8 depleted animals (Li et al., 2016). As in our companion article (Krebs et al., 2016), only one SHIVenv clone in the pool established infection. This study shows heightened replication efficiency of the viruses establishing infection and disease, and suggests that preferential infection from the pool was related to low Env glycosylation, whereas those viruses showed no differences in replicative fitness, host cell entry efficiency, sensitivity to entry inhibitors, or receptor affinity (Krebs et al., 2016).

Until recently (Krebs et al., 2016), envelopes currently utilized in SHIVenv constructs were isolated from chronically infected humans. These chronic-derived envelopes were likely exposed to extensive humoral pressure within the patients during disease. A monovalent SHIV derived from a single chronic *env* gene may fail to represent the Env in the transmitted HIV-1, which may have been "selected" from inoculating HIV-1 population and may have a distinct phenotype (Zhu et al., 1993; Keele et al., 2008; Salazar-Gonzalez et al., 2009; King et al., 2013). It is also possible that the chronic envelopes used in current SHIVs have different sensitivities to neutralizing antibodies, cell mediated responses, and/or innate immunity than SHIVenv derived from acute HIV-1 infections (AHIs) (Mikell et al., 2011; van Gils et al., 2011; Moore et al., 2009). Thus, vaccine and other intervention studies performed in macaques may be biased by the use of challenge SHIV viruses that are monovalent, derived from a chronic Env, and fail to maintain a prolonged, pathogenic, HIV-like infection.

Despite years of research, there are no defined attributes guiding the selection of specific acute-derived HIV-1 for the development of a transmissible and pathogenic SHIVenv virus for macaques. Furthermore, success in HIV-1 *env* cloning into an SIV backbone and subsequent production of this chimeric virus in human or macaque cell cultures does not guarantee subsequent infection of macaques let alone prolonged viremia and disease (Chen et al., 2000; Gautam et al., 2007; Reimann et al., 1996b). For our studies, *env* genes were derived from single genome amplifications from 16 acute HIV-1 infections (AHI) (Keele et al., 2008; King et al., 2013). We have cloned these 16 acute HIV-1 *env* genes to produce chimeric HIV-1env and SHIVenv viruses and characterized their replication kinetics on various primary T cells, macrophages, dendritic cells, and mucosal tissue (Krebs et al., 2016). Instead of pursuing a one-by-one approach, we pooled these 16 SHIVenv viruses for infection of two macaques. As described in the companion article (Krebs et al., 2016), a single SHIV clone, SHIVenv_B3 established infection in macaque m328-08. This AHI Env in this SHIVenv clone had no selective advantage over the other 15 SHIVenv clones based on replicative fitness, entry efficiency, and sensitivity to CCL5 inhibition. However, the Env_B3 did have the fewest N-linked glycosylation sites and the lowest binding affinity to C-type lectins.

In this study, the plasma from m328-08 harboring SHIVenv_B3 was used to infect two other macaques (m165-05 and m349-08), one of which (m165-05) experienced a prolonged infection (> 800 days) with declining CD4 T cell counts similar to that observed in HIV-infected humans. The HIV-1 *env* from this macaque 165-05 was RT-PCR amplified at day 21, cloned back into the SHIVenv_KB9 or the SIV_E660 backbones to produce new SHIVenv virus. The m165-05 plasma at day 21, SHIVenv_KB9-B3$_{m165\text{-}05d21}$, and SHIVenv_E660-B3$_{m165\text{-}05d21}$ viruses were then used to infect 4 macaques each. Based on the collective analyses of these macaque infections, it appears that a strong humoral response in macaques may lead to rapid clearance and limit prolonged pathogenic infection by most of these R5 SHIVenv viruses. Lower Env glycan content of SHIVenv_B3 over the other strains may relate to its outgrowth at acute infection (van Gils et al., 2011; Moore et al., 2009). However, maintenance of prolonged viremia may require rapid evolution and escape from a strong neutralizing antibody and CTL response, which of course varies in each macaque. The "pathogenic" SHIVenv_B3 evolved during infection of the m165-05 but not in m349-08, and only after the day 21. The day 21 env_B3 for m165-05 was cloned into the SHIVenv_KB9 and _E660 vectors but these clonal SHIVs did not result in a pathogenic infection of macaques. We have now identified genotypic signatures in the SHIVenv_B3 of m165-05 (after day 21) which appear to be the key for HIV-like pathogenesis in macaques.

2. Results

2.1. In vitro analyses of a pool of SHIVenv viruses with envelopes derived from acute/early HIV-1 infections

The gp120 *env* coding region from the 20 AHI viruses derived from CHAVI001 were cloned into SHIVenv_KB9 by yeast recombination gap repair technology (Dudley et al., 2009). As described, AHI gp120 coding regions (CCR5-using) replace the HIV-1 gp120 sequence as part of the HIV-1 *env*_KB9 (CXCR4-using) within the SIVmac239 backbone (Krebs et al., 2016). These SHIVenv viruses were screened for optimal capsid p27 levels, reverse transcriptase activity, as well as Env glycoproteins that could mediate cell entry through CCR5 and CD4 receptors (Krebs et al., 2016). These viruses displayed minimal but similar replicative capacity on various human CD4+/CCR5+ cell lines, human, or macaque PBMCs. As previously described, SHIVenv propagation on human cell lines and even macaque PBMCs was not indicative (and even counter-indicative) of subsequent infection and replication in macaques (Chen et al., 2000; Gautam et al., 2007; Reimann et al., 1996b).

Based on these functional screens, 16 SHIVenv_KB9_B were pooled at equal infectious units (500 IU per virus) and then used to inoculate two *Mamu-A*01-*, *B*08-*, *B*17-*, naïve Indian-origin rhesus monkeys. It is important to note that the SHIVenv inoculations were intended to establish a persistent infection in macaques and not to overwhelm the macaque with virus leading to extreme acute infection and rapid mortality. With the first inoculation with the pooled SHIVenv, one of the two animals (m328-08) developed viremia of 2×10^7 RNA copies/ mL by 3 weeks post-inoculation and reached a viral load set point $\sim 10^4$ RNA copies/mL by 6 weeks after inoculation (Fig. 1A) (Krebs et al., 2016). However, viral clearance was evident in m328-08 by day 100 (Fig. 1A).

One mL of plasma and cells harvested from animal m328-08 at day 19 and at day 41 post-infection were inoculated into animals m349-08 and m165-05, respectively. Both animals developed viremia greater than 10^7 RNA copies/mL within 3 weeks of infection (Fig. 1B). Animal m349-08 controlled viremia to below the limit of detection (500 RNA copies per mL) by day 100 post-infection. Viral RNA ($\sim 10^3$ copies/mL) was detectable again at 140 and 175 days in m349-08 but no further viremia was observed through to day 400. In contrast, animal m165-05 established a viral load set point of approximately 10^4 copies/mL which persisted in the 10^3–10^4 range for over 750 days post infection (Fig. 1B). A persistent two years infection of a macaque with a CCR5-tropic SHIVenv is rare. In addition, the SHIV-infected m165-05 showed evidence of disease progression with a drop in total 50% CD4+ T cell counts from 1968 cells/ mm^3 prior to infection to 802 cells/ mm^3 by day 770 (Fig. 2A). CD4 T cell declines were not observed in the m328-08 macaque exposed to the SHIVenv pool or in the m349-09 macaque exposed to the day 19 plasma/cells (peak viremia) from m328-08