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A genetically engineered live-attenuated simian–human immunodeficiency virus that co-expresses the RANTES gene improves the magnitude of cellular immunity in rhesus macaques

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Abstract

Regulated-on-activation-normal-T-cell-expressed-and-secreted (RANTES), a CC-chemokine, enhances antigen-specific T helper (Th) type-1 responses against HIV-1. To evaluate the adjuvant effects of RANTES against HIV vaccine candidate in SHIV-macaque models, we genetically engineered a live-attenuated SHIV to express the RANTES gene (SHIV-RANTES) and characterized the virus's properties *in vivo*. After the vaccination, the plasma viral loads were same in the SHIV-RANTES-inoculated monkeys and the parental *nef*-deleted SHIV (SHIV-NI)-inoculated monkeys. SHIV-RANTES provided some immunity in monkeys by remarkably increasing the antigen-specific $CD4^+$ Th cell-proliferative response and by inducing an antigen-specific IFN-γ ELISpot response. The magnitude of the immunity in SHIV-RANTES-immunized animals, however, failed to afford greater protection against a heterologous pathogenic SHIV (SHIV-C2/1) challenge compared to control SHIV-NI-immunized animals. SHIV-RANTES immunized monkeys, elicited robust cellular $CD4^+$ Th responses and IFN-γ ELISpot responses after SHIV-C2/1 challenge. These findings suggest that the chemokine RANTES can augment vaccine-elicited, HIV-specific $CD4^+$ T cell responses.

Keywords: SHIV; HIV-1; SIV; RANTES; Adjuvant; Vaccine; Chemokine

Introduction

Development of an effective vaccine against HIV-1 has been a major priority to control the worldwide AIDS epidemic. To achieve this goal, several approaches are being tried. Chimeric simian and human immunodeficiency virus (SHIV) clones containing the HIV-1 *env* genes on a simian immunodeficiency virus (SIV) are useful models for HIV-1 vaccine development, because SHIVs are readily infectious to macaque monkeys, and show induction of immune responses to HIV-1 Env. We previously reported the *in vivo* properties of SHIV-NM3rN (derived from HIV-1 NL432 and SIV mac239) with deletion in the *vpx*, *vpr*, and/or *nef* genes (Igarashi et al., 1998). These gene-deleted SHIVs are candidates for vaccines against HIV-1 because attenuated SHIVs can induce anti-HIV-1 humoral and cell-mediated immunity in monkeys without AIDS-like diseases (Haga et al., 1998; Kuwata et al., 2001). Moreover, the monkeys immunized with the *nef*-deleted SHIVs (SHIV-NI) were protected from a challenge with a heterologous pathogenic SHIV (Enose et al., 2002; Ui et al., 1999). Since live-attenuated virus vaccines mimic natural exposure to infectious disease, these vaccines effectively induce very durable humoral and cell-mediated immunity. Live-attenuated SIV/SHIVs have been shown to be effective vaccines in macaque models. Although safety considerations have limited trials of live-attenuated HIV vaccines in humans (Baba et al., 1999; Gundlach et al., 2000), determining the mechanism of protection of live-attenuated

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lentiviruses in primates may help to develop HIV-1 vaccines (Koff et al., 2006; Miller and Abel, 2005). In general, the immunogenicity of live-attenuated vaccines tends to increase with increasing virulence (Johnson and Desrosiers, 1998). Therefore, in attenuating a live virus, there is a trade-off between safety and immunogenicity. One promising new strategy to improve immunogenicity of live-attenuated vaccines is to genetically engineer a virus to co-express an immunostimulatory agent such as a cytokine adjuvant. Several studies have demonstrated that insertion of a cytokine in gene-deleted live-attenuated SIV/SHIVs could boost its immunogenicity and enhance its protection ability (Giavedoni et al., 1997; Stahl-Hennig et al., 2003). This would make it possible to obtain a higher level of immunogenicity from safer, less virulent strains.

Chemokines are key players in eliciting immune responses against viral infections, by selective activation of a subpopulation of immune cells and by attracting these cells to the site of infection (Luster, 1998). Regulated-on-activation-normal-T-cell-expressed-and-secreted (RANTES) is a CC-chemokine and a natural ligand for CC-chemokine receptors (CCRs) 1, 3, 4, and 5. CCR5, the main receptor of RANTES, is expressed mainly on subsets of monocytes, macrophages, NK cells, and T lymphocytes that are predominantly associated with T helper type-1 (Th1) responses (Bonecchi et al., 1998; Loetscher et al., 1998; Sallusto et al., 1998). An immune response polarized toward a more Th1 response is associated with a reduced viral load and non-progression of disease in HIV-1 infection (Imami et al., 2002). RANTES has been found to enhance cellular immune responses resulting in a more effective immune-modulating effect against HIV-1-related virus in rodent and monkey models (Frauenschuh et al., 2004; Kim et al., 1998; Waterman et al., 2004; Xin et al., 1999). In addition, infection of macaques with a live-attenuated SIV induced the production of CC-chemokines, and the up-regulation of CC-chemokines was found to be associated with the sterilizing immunity generated by the vaccine (Ahmed et al., 1999; Gauduin et al., 1999; Heeney et al., 1998). Moreover, RANTES has been shown to directly inhibit HIV-1 replication *in vitro* (Alkhatib et al., 1996; Cocchi et al., 1995). RANTES blocks or down-modulates CCR5 *in vitro*, which leads to suppression of CCR5-tropic (R5-tropic) HIV-1 infections (Proudfoot et al., 1999). These results make RANTES an attractive candidate as an immune adjuvant.

In order to test the adjuvant effects of RANTES in SHIV-macaque models, we previously genetically engineered a SHIV to co-express the human RANTES gene (SHIV-RANTES) and characterized its properties *in vitro* (Shimizu et al., 2006). In a previous *in vitro* study, along with the replication of SHIV-RANTES, RANTES was detected in the culture supernatant at a maximum level of 98.5 ng/ml in SHIV-RANTES-infected human $CD4^+$ M8166 cells. The expressed RANTES down-regulated the expression of CCR5 on PM1 cells, and suppressed the replication of BaL, a R5-tropic HIV-1.

In this study, we demonstrate that SHIV-RANTES provided effective cellular immunity in inoculated monkeys by inducing an antigen-specific proliferation of lymphocytes and by increasing an antigen-specific gamma interferon (IFN-γ) enzyme-linked immunospot (ELISpot) response.

Results

Immunization of SHIV-NI and SHIV-RANTES

A recombinant SHIV was engineered to express RANTES (SHIV-RANTES) in place of *nef* in SHIV-NI (Fig. 1A). To investigate the *in vivo* properties of a SHIV-RANTES, four rhesus monkeys (MM426, MM427, MM430, and MM431) were intravenously inoculated with 10^5 tissue culture 50% infectious dose ($TCID_{50}$) of SHIV-RANTES, and three animals (MM428, MM432, and MM434) were inoculated with 10^5 $TCID_{50}$ of SHIV-NI as a control. All monkeys were viremic within 2 weeks post inoculation (wpi) (Fig. 2A). The SHIV-NI and SHIV-RANTES inoculation induced systemic infection with similar peak plasma viral RNA (vRNA) levels, reaching 10^3 to 10^4 RNA copies/ml at 2 to 4 wpi and falling to almost below the detection limit by 8 wpi (Figs. 2A and B). This was also the case for two monkeys (MM346 and MM349) that were inoculated with the same dose of SHIV-NI in a previous report (Shimizu et al., 2005). The infectious viruses were transiently re-isolated at 1 to 6 wpi from co-culture of M8166 cells with PBMCs (data not shown). The stability of the RANTES gene in virus isolated from the vaccinated macaques was analyzed by

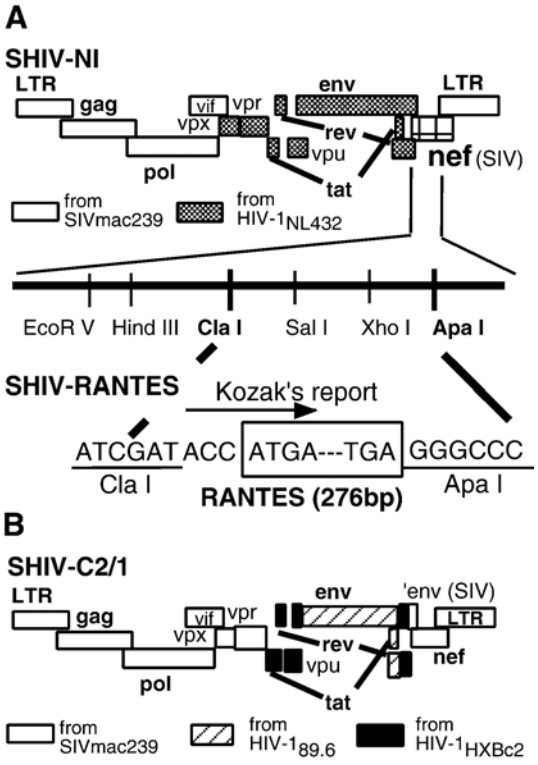

Fig. 1. Genetic structures of SHIVs used in this study. (A) The parental SHIV-NI and SHIV-RANTES. SHIV-NI has some unique restriction enzyme sites in place of the *nef* gene of SHIV-NM3rN. SHIV-NM3rN was constructed from HIV-1 NL432 (shaded regions) and SIV mac239 (white regions). The *Cla*I and *Apa*I region of SHIV-NI was replaced by the human RANTES gene (Shimizu et al., 2006). (B) The challenge virus SHIV-C2/1. SHIV-C2/1 (GenBank accession number AF217181) was generated by *in vivo* passage of SHIV-89.6 through cynomolgus monkeys (Shinohara et al., 1999). SHIV-89.6 was constructed from HIV-1 HXBc2, HIV-1 89.6, and SIV mac239.