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Immunogenicity of HIV-gag Virus-Like Particles in Rhesus

Macaques by intra-nasal administration.

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Abstract

The vast majority of new HIV infections worldwide are acquired via the genital mucosa, and women account for close to 50% of them. Therefore, development of vaccination strategies to elicit systemic and mucosal immune response represent a major goal in the HIV vaccine field.

In the present study, female rhesus macaques were immunized with HIV-Gag Virus-Like Particles (HIV-VLPs) administered as a sequential combination of mucosal (intra-nasal) and systemic (intra-muscular) routes, according to homologous or heterologous prime-boost schedules.

The results show that the sequential i.n. and i.m. administration of HIV-VLPs is able to elicit humoral immune response at systemic as well as vaginal level.

This represents the first study aiming at evaluating the immunogenicity in Rhesus Macaques of HIV-VLPs administered by i.n. and i.m. routes and shows the possible application for inducing humoral response at systemic as well as vaginal level.

The vast majority of new HIV infections worldwide are acquired via the genital mucosa, and women account for close to 50% of them ³⁷. The development of vaccination strategies able to elicit protective systemic and mucosal immune response represent a major goal in the HIV vaccine field, possibly providing a crucial method for halting the spread of HIV/AIDS.

Mucosal secretory immunoglobulin A (sIgA) specific for HIV-1 envelope glycoproteins is consistently detected in seropositive subjects ^{1,14} and has been strongly associated with protection from HIV-1 infection in uninfected individuals having unprotected sexual intercourse with HIV-1-seropositive partners ^{13,23,24,27}. Furthermore, passive administration of the gp120-directed human neutralizing monoclonal antibody b12 has been shown to be highly effective in protecting monkeys from a vaginal challenge when delivered intravenously ²⁸ or intravaginally ^{12,38}.

Considering these epidemiological and experimental evidences, it seems reasonable to believe that specific mucosal immunity is extremely relevant for controlling the primary HIV-1 infection. Intra-nasal immunization has been shown to be effective for protection against infectious respiratory diseases such as influenza ^{2,22,31,33,36,41}. However, the effectiveness of mucosal immunization often relies upon co-administration of appropriate adjuvants that can initiate and support the transition from innate to adaptive immunity (recently reviewed in ¹⁹). In addition to adjuvants, particulate antigens (e.g. virus-like particles, VLPs) have been shown to be advantageous for intra-nasal immunization, given that efficiently target antigen-presenting cells (APCs) and facilitate the induction of potent immune responses ^{7,9-11,16,17,29,32,39,41,42}.

HIV-VLPs have been developed in our laboratory expressing the whole HIV gp120/140 envelope protein derived from an Ugandan clade A field isolate ^{4-6,34,35,40}, and the elicitation of immune response at systemic as well as mucosal (vaginal and intestinal) level has been evaluated in mice by intra-peritoneal as well as intra-nasal administration ^{7,8,11}. In particular, the mucosal immunogenicity of such HIV-VLPs has been evaluated also comparing a homologous (VLP+VLP) and heterologous (DNA+VLP) prime-boost strategy by intra-nasal administration, in an adjuvanted formulation ⁷.

In the present study, the immunogenicity of HIV-VLPs has been evaluated in Rhesus Macaques immunized with HIV-Gag Virus-Like Particles (HIV-VLPs) administered as a sequential combination of

mucosal (intra-nasal) and systemic (intra-muscular) routes, according to homologous or heterologous prime-boost schedules.

24 female Rhesus Macaques were equally divided in four experimental arms and immunized by intra-nasal route as described in Fig. 1. Group 2 and 3 were immunized using a “*homologous*” prime-boost protocol (VLP prime + VLP boost) in the absence (Group 2) or in the presence (Group 3) of the Eurocine L3 nasal lipid adjuvant. Group 4 was immunized using a “*heterologous*” prime-boost protocol (DNA prime + VLP boost) in the presence of Eurocine L3 and N3 adjuvants. Group 3, 22 weeks after the last intra-nasal (i.n.) administration, received two further boosting doses of VLPs by intra-muscular (i.m.) route. Group 1 was the control group administered with adjuvants. VLPs expressing a HIV gp120 from an Ugandan clade A field isolate and DNA plasmid expressing HIV-1 gp160/rev, as well as Eurocine N3 and L3 nasal lipid adjuvants, have been previously described^{5,7,8,11,15,18,20,21,30}.

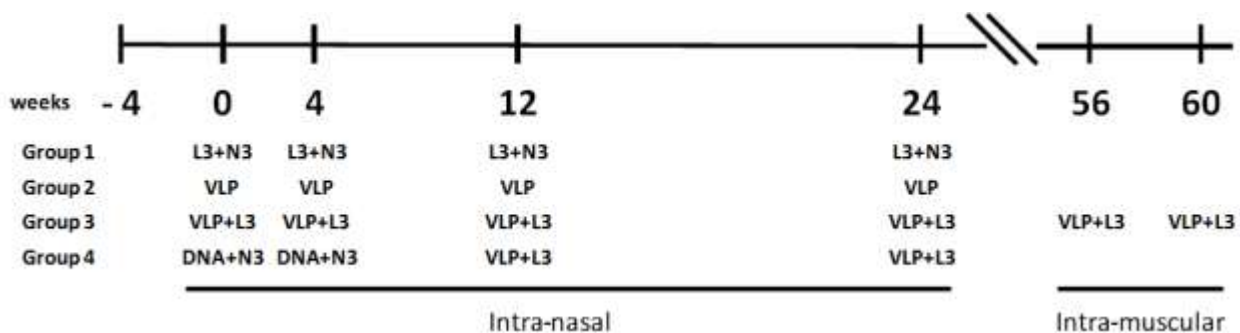


Fig. 1 Immunization scheme in NHPs. Six animals per group were immunized as described at indicated weeks.

Sera were collected from 10 ml of whole blood one week before and one week after each antigen administration and ELISA tests were performed on microwell plates coated with recombinant HIV gp120 or p24. The data show that intra-nasal administration of HIV-VLPs, neither homologous nor heterologous prime-boost protocols, does not elicit a measurable serum anti-Env or anti-Gag Ab titers (Fig. 2A).

Nevertheless, the i.n. administration protocol efficiently primes the immune system which, 6 months after the last i.n. boost, responds more swiftly to the i.m. administration in the Group 3 (Fig. 2B). In particular, evaluating the individual animals in such Group, it is possible to identify best responders for both Env and Gag (#4642 > 4635 > 4636) (Fig. 2C and D).

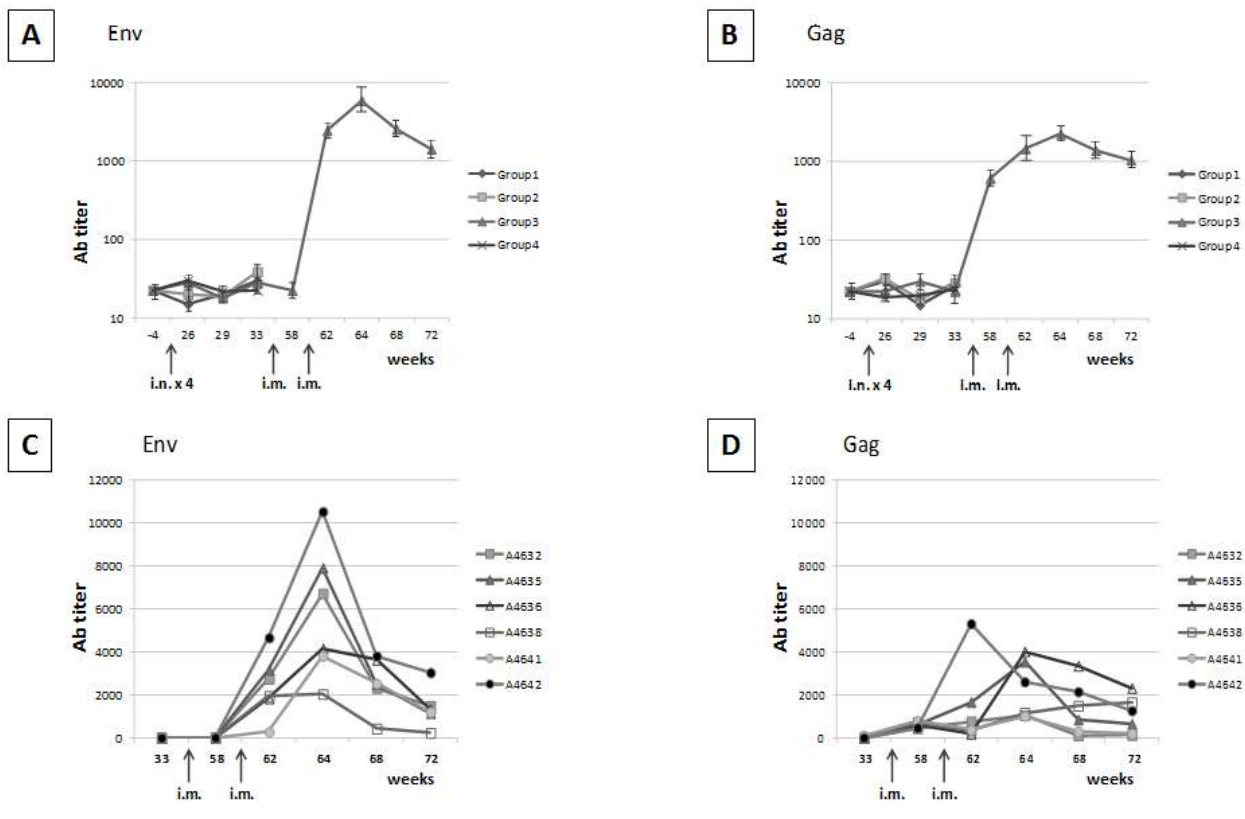


Fig. 2 Systemic immune response. Specific anti-env and anti-gag immune responses in serum of immunized animals were evaluated by ELISA. The average of Ab titers in each group is shown in tables A and B. The Ab titer for each animal in the Group 3, after the two i.m. immunizations, is shown in tables C and D.

Vaginal washes were collected at the same days as for the serum and ELISA tests were run in parallel.

The data show that intra-nasal administration of HIV-VLPs, neither homologous nor heterologous prime-boost protocols, does not elicit a measurable mucosal titers (data not shown); however, it primes the mucosal immune system which, 6 months after the last i.n. boost, is able to respond to the i.m. administration. The effect is evident in 2/6 animals in the Group and appears to be selective for Env (Fig. 3). Furthermore, antibody titers elicited by the two i.m. administrations of VLPs do not appear to be sufficient to show HIV neutralization nor ADCC activity (data not shown).

The results obtained in NHPs in the present study by i.n. administration of VLPs are in contrast to those obtained in mice⁷, however this could be either due to the administered dose (i.e., too low in NHPs) or to lower permeability to antigens of the nasal epithelium in Macaques. Indeed, our data are in agreement with results from others who have previously shown the lack of immune response in NHP by i.n.

administration using different vaccine delivery systems and suggesting that such observation is not vaccine-related^{3,25,26}.

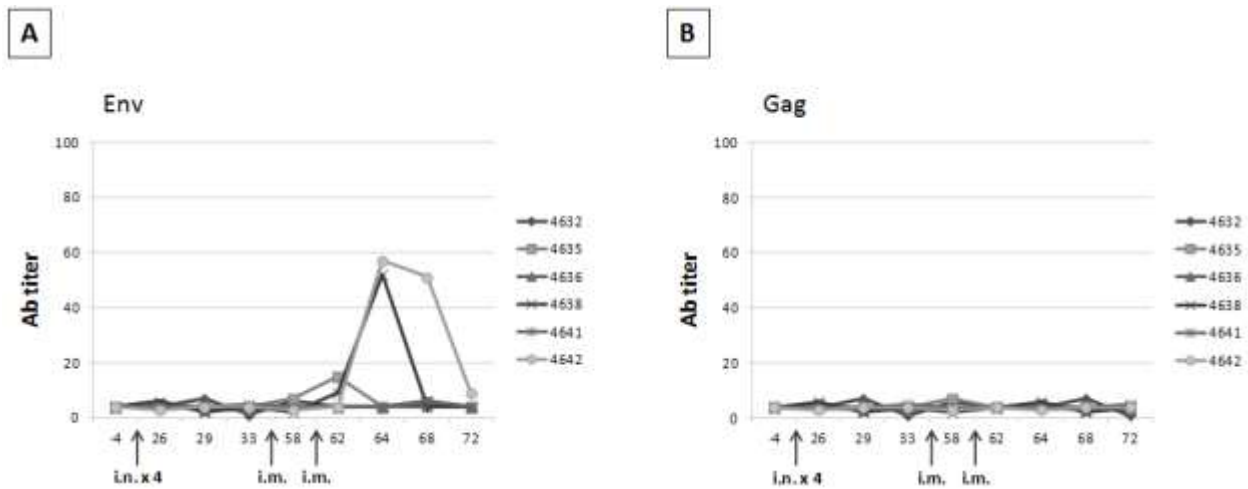


Fig. 3 Vaginal immune response. Specific anti-env and anti-gag immune responses in vaginal washes of immunized animals were evaluated by ELISA. The Ab titer for each animal in the Group 3 is shown in tables A and B.

In conclusion, the described NHP preclinical trial confirms the elicitation of specific immune response by HIV-VLPs and shows that the i.n. administration, in such animal model, is only able to prime to mucosal immune system for subsequent boosting doses. In agreement with data from other Groups, indeed, the NHP animal model does not appear to be appropriate to verify the effectiveness of the intra-nasal route for vaccine administration. This must be taken into consideration for future pre-clinical vaccine evaluations.

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