Brief Communication

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# HIV-1 vaccine components facilitate the production of virus-like particles (VLPs) and the presentation of envelope proteins that reveal broadly neutralizing epitopes.

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## ABSTRACT

Developing a preventive vaccination is hampered most by the sequence variety of HIV-1. Trialists are currently testing Mosaic (Mos) antigens, which are made up of artificially scrambled epitopes from different strains of HIV-1 (NCT03964415). The Mosaico vaccine includes vectors that mediate gene transfer and production of the membrane-anchored Env-variant Mos2S.Env in addition to adenovirus vectors encoding variations of the Mos.Gag-Pol and soluble Mos.Env proteins. Therefore, we investigated whether the production of virus-like particles (VLPs) is mediated by the expression of mosaic Gag. The Mos1.Gag- and Mos2.Gag-VLP-formation was easily identified using electron microscopy and Western blot analysis. Co-expression of Mos2S. Env and both mosaic Gag variants resulted in the integration of Env into Gag-formed VLPs. Mos2S.Env-decorated VLPs were shown to display the corresponding neutralization-sensitive target epitopes when a panel of broadly neutralizing antibodies (bNAbs) was used in a VLP-capture test. This provides fresh insights for developing HIV vaccines in the future.

## KEYWORDS

Vaccine development ,Mosaic HIV-1 antigens p55-gag virus-like particles, Broadly neutralizing antibodies, PiggyBac transposon vectors, Suspension cell lines

## INTRODUCTION

It is vitally necessary to create a preventive vaccination in order to fight the HIV/AIDS epidemic. A perfect vaccination would stimulate humoral as well as cellular immunity. However, the development of a vaccine is severely hampered by the great genetic heterogeneity of HIV-1 (Gaschen et al., 2002; Ndung'u and Weiss, 2012). To reduce the number of infected cells and, consequently, the viral load, a robust T cell response—especially one that involves CD8+ cytotoxic T lymphocytes (CTLs)—must be triggered (Kiepiela et al., 2007; Janes et al., 2017; Collins et al., 2020). Furthermore, in order to prevent virus cell entrance, a strong vaccination must stimulate the production of broadly neutralizing antibodies (bNAbs) against neutralization-sensitive epitopes found in the viral envelope glycoproteins (Env) (Zolla-Pazner et al., 2014;

So-called mosaic (Mos) HIV antigens, which are composed of artificially shuffled epitope sequences coming from several HIV variations, were created to increase coverage of potential T and B cell epitopes originating from diverse HIV variants (Fischer et al., 2007). When rhesus macaques were vaccinated with an adenovirus vector containing the antigen-encoding genes Mos.Gag, Mos.Pol, and Mos.Env, the results showed that the antigen-specific T cell responses were more extensive and varied than when utilizing consensus or naturally occurring sequences (Barouch et al., 2010). These results served as the foundation for the ongoing Mosaico clinical trial (NCT03964415). In addition to soluble Env proteins acting as booster vaccine subunit components, the The adenovirus vector components Ad26.Mos1.Gag-Pol, Ad26.Mos2.Gag-Pol, Ad26.Mos1.Env, and Ad26.Mos2S.Env are part of the Ad26. Mos4 tetravalent vaccination (Ad26.Mos4) for HIV (Baden et al., 2020). Mos2S.Env, also known as C4D7, is membrane-anchored because it possesses a transmembrane region, in contrast to the soluble Mos1.Env. When compared to its full-length counterpart, the shortened cytoplasmic tail (CT) of Mos2S.Env was demonstrated to enhance surface expression (Langedijk et al., 2019, 2021). The ability of the artificial mosaic Gag variations to promote the generation of virus-like particles (VLPs), as seen with naturally occurring Gag precursor proteins (Gheysen et al., 1989; Konnyu et al., 2013), has not yet been investigated. Following the administration of the tetravalent

vaccine Ad26.Mos4.HIV, vaccination recipients may develop VLPs. VLPs significantly increase cellular.

To evaluate the capacity of mosaic antigens to generate vector lip products (VLPs), we generated viable human suspension cell lines that expressed either Mos1.Gag and Mos2.Gag alone or in combination with Mos2S.Env. Transposon vectors generated from piggyBac were created and then transfected 293-F cells in order to accomplish this. This method was used since transposon vectors have been demonstrated in the past to facilitate the quick establishment of recombinant cell lines that produce particles derived from viruses (Lynch et al., 2010; Berg et al., 2019; van Heuvel et al., 2021). Cell-free supernatants were pelleted using ultracentrifugation, and the resulting pellets were submitted to Western blot and electron microscopic examination to determine if bald and Env-decorated VLPs were generated.

Additionally, bNAbs were used in VLP capture tests to precipitate VLPs that were likely to have epitopes responsive to neutralization. VLPs with precipitated env-positive.

## RESULTS

The initial evidence that the expression of both Mos1.Gag and Mos2.Gag, respectively, in the absence or presence of Mos2S. Env were able to mediate VLP-formation came from transient transfection experiments using HEK293T cells and the described transposon vectors containing mos1. gag, mos2.gag, and mos2s.env. Using a p24-specific ELISA, it was discovered that cell-free supernatants three days after transfection contained up to 110 ng/mL of gag (data not shown). Transposon-mediated gene transfer was used to quickly establish cell lines that persistently expressed the mosaic HIV genes, demonstrating the formation of VLPs and facilitating in-depth study. The very active piggyBac transposase is made possible by CMVhyPBase, as shown in Fig. 1 (Yusa et al., 2011). For each mosaic, the transposon constructions PB-Mos1.Gag-IpW and PB-Mos2.Gag-IpW encode. gag genes connected to puromycin resistance gene expression. Vector donor PB-Mos2S.Env consists of the hygromycin resistance gene connected to the mosaic env gene. The suspension cells of human 293-F were.

The aforementioned VLP samples were also submitted to Western blot-analysis employing polyclonal antibodies directed against the surface unit gp120-SU of HIV-1 Env in order to investigate the possible inclusion of Mos2S.Env into mosaic Gag-formed VLPs. Negative controls were samples of uninfected 293-F cells and cells that expressed just Gag variations, respectively. Mos2S.Env proteins were easily and only found in VLPs made by gag- and env-transgenic cells, as shown in Fig. 3. by the cellular furin-like proteases (McCune et al., 1988; Hallenberger et al., 1992; Decroly et al., 1994). of the precursor proteins. There were no signs of Env degradation products.

The levels of Env protein found were similar. This demonstrated that Mos2S.Env was effectively incorporated into VLPs created by both mosaic Gag proteins. Remarkably, extremely few amounts of the unprocessed precursor gp140-Pr proteins were found, whereas the great majority of Env identified were fully processed, and hence soluble, gp120-SU proteins. This demonstrated the extremely effective processing.

We created a piggBac-derived transposase construct and three transposon vectors encoding Mos1.Gag, Mos2.Gag, and Mos2S.Env, respectively, to quickly establish stable human 293-F suspension producer cell lines upon co-transfection and selection in order to investigate VLP-formation mediated by mosaic gene expression. Using negative stain transmission electron microscopy (TEM) analysis, VLPs were easily identified when Mos1.Gag, Mos2.Gag, and Mos2S were expressed exclusively. These results are consistent with other studies (Chapman et al., 2017) that showed mosaic Gag sequences might induce VLPformation following expression in human cells.

Following co-transfection of cells with mosaic gag and chimeric env plasmid vectors and infection of cells with MVA vaccines, Chapman and colleagues have previously shown the in vivo formation of Env-decorated mosaic Gag VLPs.

cleaves Gag into its subunit components, a process that would be mediated by the viral protease and included in the Ad26.Mos4.HIV vaccine. On the other hand, this should have a favorable impact on the particles' immunogenicity and, thus, their potential as vaccine ingredients. In a comparative investigation using DNA/MVA vaccinations in rhesus monkeys, Ellenberger and colleagues reported on the enhanced immunogenicity of mature as opposed to immature VLPs (Ellenberger et al., 2005).

## MATERIALS AND METHODS

The transposon donor vectors and the transposase construct had nearly identical genomic designs with the vectors derived from Sleeping Beauty that we previously reported (Berg et al., 2019, 2020). The PB-CAG-DDdCas 9VP192-T2A-GFP-IRES-Neo, a generous donation from Timo Otonkoski (plasmid no.102885; Addgene, USA), provided the DNA sequences for the piggyBac ITRs. After being synthesized (GenScript, USA), the ITRs were introduced into pUC57 to create pUC57-piggyBacITRs. Using FseI restriction sites, the transgene expression cassettes of the donor vectors SB-IpW and SB-IhW, which were generated from Sleeping Beauty, were introduced into pUC57-piggyBacITRs to produce PB-IpW and PB-IhW, respectively. Using codon optimization for human and Chinese hamster ovary cells, the genes encoding Mos1.Gag, Mos2.Gag, and Mos2S.Env, respectively, were synthesized (GenScript, USA) and inserted

into pUC57. Human suspension 239-F cells (Thermo Fisher Scientific Cat #R79007, RRID: CVCL_D603, USA) were used in all investigations.

expanded in Thermo Fisher Scientific's (293F-FreeStyle-Expression-Medium, USA) serum-free formulation. In order to generate stable VLP producer cells, 293-F cells were co-transfected with the transposase construct and one of the gag-expressing transposon vectors, or both in conjunction with the transposon vector encoding Mos.2SEnv. As previously mentioned (Berg et al., 2020), 293-F cells were transfected using 1 g/L polyethylenimine (PEI; MW 40,000; Polysciences, USA) and then stable cell lines were chosen. In summary, 50 µg of plasmid DNA total was utilized for transfection, and the hyPBase construct was always used in a 1:1:4 ratio with Gag expressing transposon vectors. consistent producing cell lines that express Gag and Env were created via transfection at a 1:2:2 ratio. A moderate selection pressure was applied four days after transfection (dpt) utilizing doses of 4 µg/mL puromycin, or equivalently, 4 µg/mL puromycin combined with 50 µg/mL hygromycin. Antibiotic concentrations were continuously raised. Starting at day 21, recombinant cell lines were maintained under continuous selection pressure with 200 µg/mL hygromycin, 10 µg/mL puromycin, and 15 µg/mL puromycin, respectively. Using 250–500 mL disposable shaker flasks with vent lids (Nalgene Nunc International, USA), cultivation was done in quantities of 50–200 mL media at starting cell densities of 0.6 x 106 cells/mL. A Minitron shaker incubator with an 8% CO2 atmosphere, 37 °C, and 135 rpm was used for the incubation.

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