

SHORT REPORT

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Presenting native-like HIV-1 envelope trimers on ferritin nanoparticles improves their immunogenicity

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Abstract

Background: Presenting vaccine antigens in particulate form can improve their immunogenicity by enhancing B cell activation.

Findings: We describe ferritin-based protein nanoparticles that display multiple copies of native-like HIV-1 envelope glycoprotein trimers (BG505 SOSIP.664). Trimer-bearing nanoparticles were significantly more immunogenic than trimers in both mice and rabbits. Furthermore, rabbits immunized with the trimer-bearing nanoparticles induced significantly higher neutralizing antibody responses against most tier 1A viruses, and higher responses (but not significantly), to several tier 1B viruses and the autologous tier 2 virus than when the same trimers were delivered as soluble proteins.

Conclusions: This or other nanoparticle designs may be practical ways to improve the immunogenicity of envelope glycoprotein trimers.

Keywords: HIV-1, Envelope glycoprotein, Ferritin, Nanoparticles, Vaccine, SOSIP, BG505

Findings

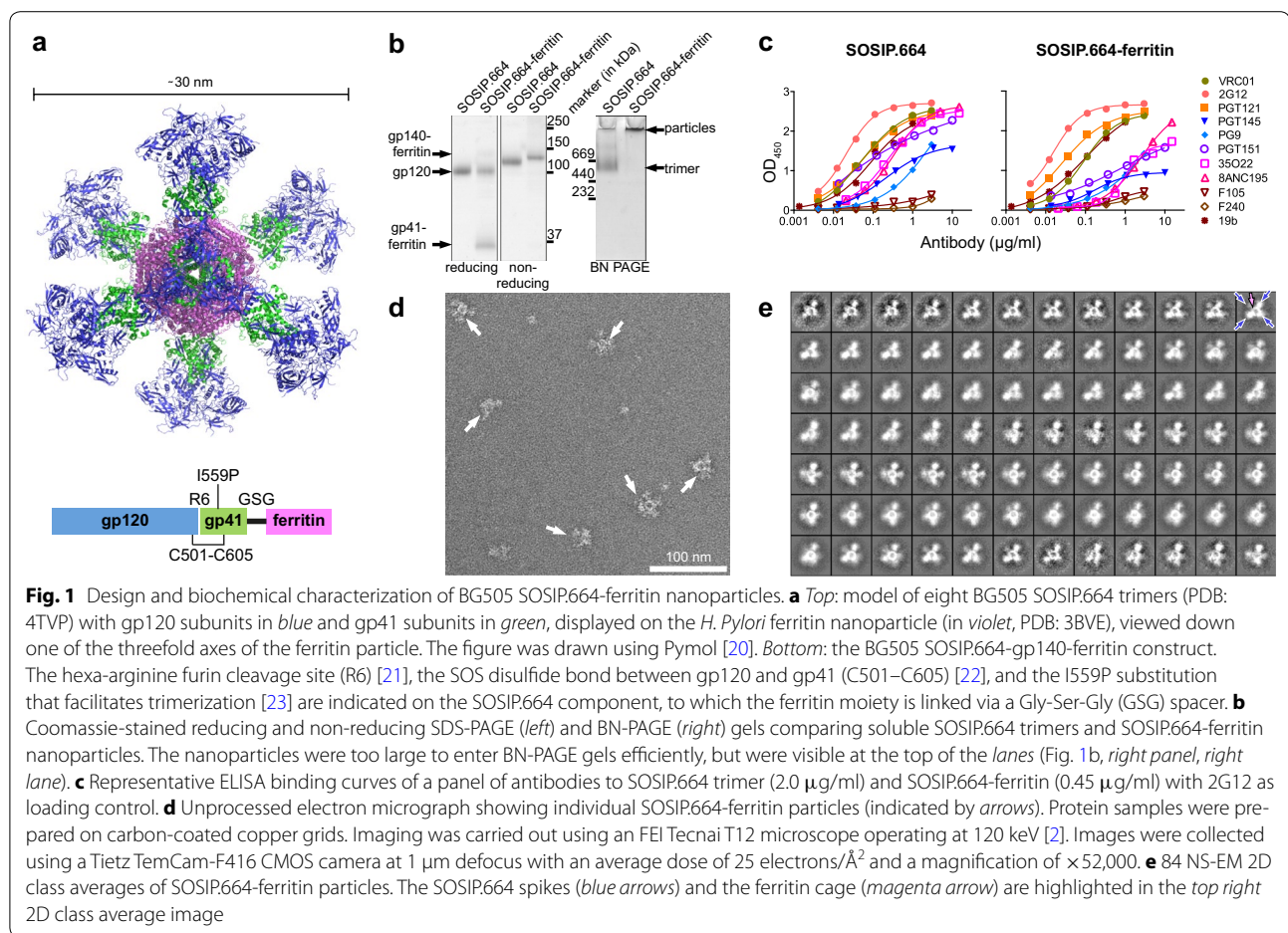
An HIV-1 subunit vaccine should induce a broad and potent neutralizing antibody (NAb) response against the envelope glycoprotein spike (Env) [1]. Soluble, stable mimics of the native spike, such as the BG505 SOSIP.664 gp140 trimer, might be good starting points for such a vaccine [2–5]. These trimers bind virtually all known broadly neutralizing antibodies (bNAbs) but almost no non-neutralizing antibodies (non-NAbs), and adopt a native-like conformation with a well-defined structure [2, 6–8]. Furthermore, unlike other gp140 proteins, soluble, adjuvanted BG505 SOSIP.664 trimers induce NAbs against the autologous, neutralization-resistant (tier 2) virus efficiently in animals [9]. Licensed subunit vaccines against viral pathogens, such as hepatitis B virus and human papillomavirus, are however particulate antigens

[10]. The greater size and the capacity for multivalent antigen presentation and B cell receptor cross-linking provide such particulate vaccines with advantages over soluble proteins for inducing antibody responses [11]. For example, fusing eight influenza hemagglutinin (HA) trimers or engineered HA stem antigens to *Helicobacter pylori* ferritin greatly improved NAb responses against influenza in animals [12, 13].

Modeling showed that *H. Pylori* ferritin (GenBank accession no. NP_223316) could potentially present eight BG505 SOSIP.664 trimers. Therefore we fused the ferritin N-terminus, starting from Asp5, to the SOSIP.664 C-terminus, separated by a Gly-Ser-Gly (GSG) linker (Fig. 1a). The SOSIP.664-ferritin plasmid was co-transfected into 293F cells with a furin plasmid to maximize trimer cleavage and ensure it adopts a native conformation [14]. To select for antigenically and structurally well-folded Env proteins, the secreted nanoparticles and control trimers were purified using PGT145 bNAb-affinity chromatography [15]. Judged by BN-PAGE and SDS-PAGE analysis

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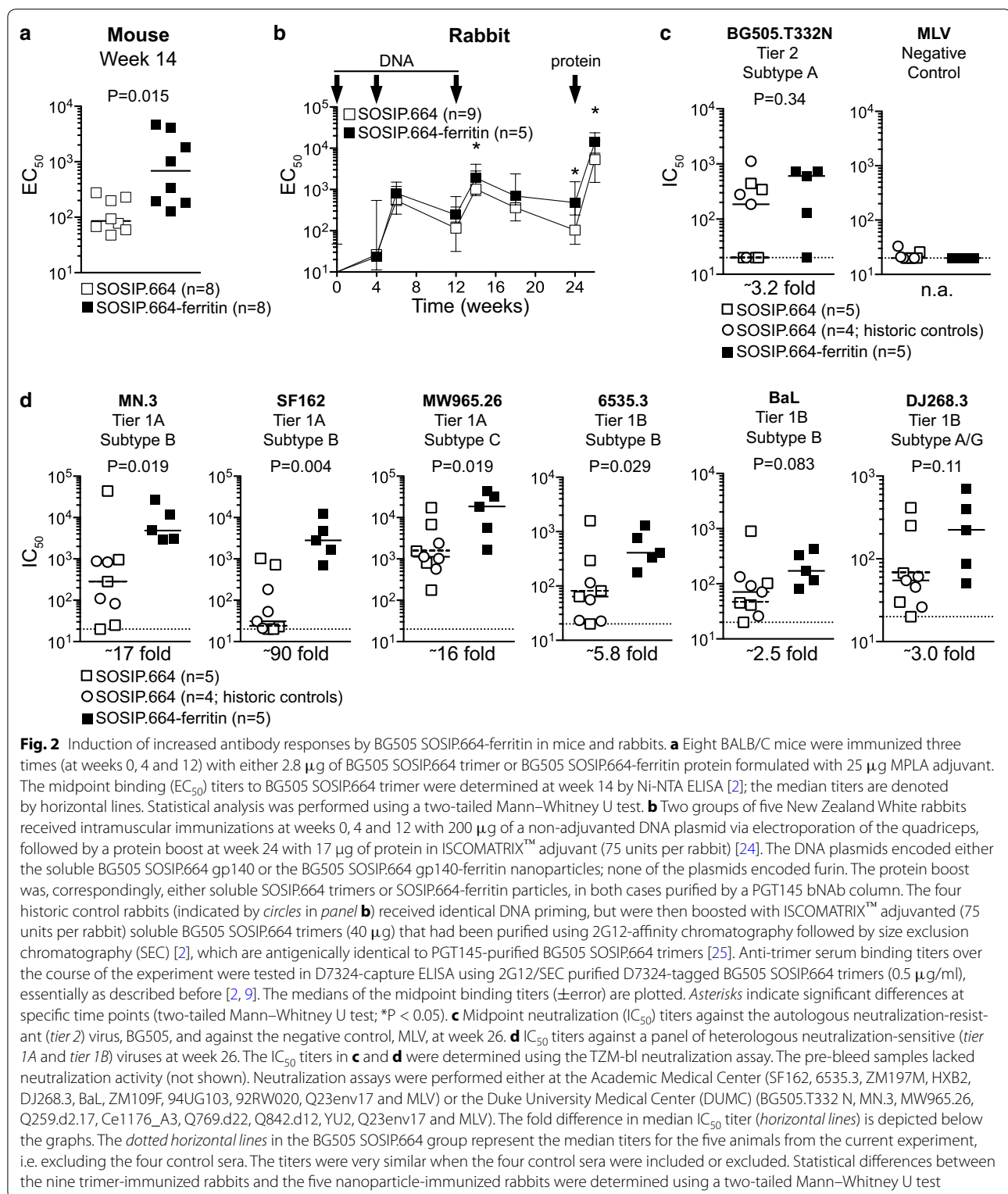
followed by Coomassie staining this purification method yielded highly pure (>95 % purity) SOSIP.664 trimer and SOSIP.664-ferritin protein preparations (Fig. 1b). SDS-PAGE also confirmed that the SOSIP.664 component of the nanoparticles was cleaved efficiently between gp120 and gp41 (Fig. 1b, left panel).

The antigenic structure of SOSIP.664 trimers and SOSIP.664-ferritin was compared using ELISA. Proteins were captured using *Galanthus nivalis* lectin and probed with bNAbs and non-NAbs (Fig. 1c). Several bNAbs that bind to distinct Env epitopes (VRC01, PGT121, PG9) showed similar binding to SOSIP.664 and SOSIP.664-ferritin, moreover non-NAbs (F105 and F240) displayed similarly poor reactivity with both proteins (Fig. 1c). We did observe lower affinity of gp120/gp41 interface (8ANC195, 35O22 and PGT151) and gp41 (3BC315) bNAbs for SOSIP.664-ferritin, which might be explained by steric hindrance of neighboring trimers on the nanoparticle (Fig. 1c).

The purified nanoparticles were analyzed by negative stain electron microscopy (NS-EM). More than 70 % of the particles on the EM grid resembled ferritin cages

with protruding spikes that were 30–40 nm in diameter (Fig. 1d). When single particles were automatically picked and processed as described elsewhere [2], 2D class averages representing views along the three- and fourfold symmetry axes suggested that 65–80 % of the SOSIP.664-ferritin particles were fully decorated with Env trimers (three and four spikes visible, respectively) (Fig. 1e). The lack of views along the twofold symmetry axis (i.e. six spikes visible) may be a result of the immobilization on the EM grid or flexibility of the GSG-linker that affects the alignment of the particles and visualization of each Env trimer.

We first immunized mice (approved by the AMC animal ethics committee: DMB-102836; $n = 8$ mice per group) to compare the antibody response of SOSIP.664-ferritin nanoparticles with soluble (i.e. monovalent) SOSIP.664 trimers. The anti-trimer binding responses were eightfold higher in mice vaccinated with nanoparticle-displayed trimers compared to soluble trimers (medians: 86 vs. 686; $P = 0.015$) (Fig. 2a). We next immunized rabbits (approved by the Covance Institutional Animal Care and Use Committee (IACUC):



0082-14; n = 5 rabbits per group), using a triple DNA-prime, protein-boost regimen (Fig. 2b). Given the limited group sizes and the large spread in neutralization titers

generally observed in other HIV-1 vaccination studies [9], we included historic control sera from four rabbits to increase the statistical power of this study. These rabbits

were immunized with the soluble trimers in an independent experiment using the same DNA prime + protein boost protocol (approved by the Covance IACUC: 0001-14; n = 4 rabbits per group; unpublished results). As expected, the anti-trimer binding antibody responses rose and fell between immunizations, and were boosted by the protein-only immunization [9, 16]. The titers were two- to threefold higher at several time points for the rabbits given SOSIP.664-ferritin nanoparticles compared to the soluble trimers. Although the improved immunogenicity was less pronounced in rabbits compared to mice, it is consistent with other observations showing the benefits of particulate antigen presentation [12, 17, 18] (Fig. 2b).

We used the TZM-bl cell neutralization assay and viruses from different clades to assess the serum NAb titers 2 weeks after the protein boost in rabbits [19]. Sera from 4/5 rabbits given the SOSIP.664-ferritin nanoparticles neutralized the autologous BG505.T332 N tier 2 virus, and the median titer in this group was higher than in the soluble trimer group (603 vs. 186). However, because of the small group sizes, the difference was not statistically significant ($P = 0.34$) (Fig. 2c). The NAb titers against heterologous tier 1 viruses were also higher in the rabbits that received SOSIP.664-ferritin nanoparticles (Fig. 2d). Median NAb titers against tier 1A viruses were 10- to 90-fold higher in the nanoparticle group: MN.3 (4,857 vs. 282; $P = 0.019$); SF162 (2,799 vs. 31; $P = 0.004$); MW.965 (18,563 vs. 1,127; $P = 0.019$). For the more resistant tier 1B viruses the titers were also higher, although this did not reach statistical significance in all cases: 6535.3 (472 vs. 82; $P = 0.029$); BaL (171 vs. 71; $P = 0.083$); DJ286.3 (195 vs. 64; $P = 0.11$). The tier 1B viruses HxB2, Q23env17, ZM109F and ZM197M and the tier 2 viruses 94UG103, 92RW020, Q259.d2.17, Q769.d22, Q842.d12 (all clade A), YU2 (clade B) and Ce1176_A3 (clade C) were not neutralized by any rabbit sera (data not shown).

Conclusions

We conclude from this exploratory study that the nanoparticle display of SOSIP.664 trimers improves the magnitude of the overall antibody response and neutralization breadth at the tier 1 level. We are seeking to solve the substantial problem of inducing a bNAb response (at the tier 2 level) by improving the design of native-like trimers such as BG505 SOSIP.664 and/or how they are used as immunogens. If and when this goal is achieved, the superior immunogenicity of a particulate antigen presentation should be valuable.

Authors' contributions

KS conceived the project and carried out experiments, analyzed the data and wrote the manuscript. GO and ABW performed the electron microscopy

experiments, analyzed the data and co-wrote the manuscript. JB, CLB and DCM carried out and interpreted neutralization experiments. TvM and MS purified proteins, performed mice vaccinations and performed ELISA experiments; JPM co-wrote the manuscript. RWS conceived the project, participated in the experimental design and wrote the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

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