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Visualization of CombiHIVvac Vaccine Particles Using Electron Microscopy

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A candidate vaccine CombiHIVvac is developed; presently the clinical phase I trial has been completed successfully. CombiHIVvac combines the conserved polyepitope immunogens approaches in a novel self-adjuvanted microparticle concept. The artificial TBI (T cell and B cell immunogen) polypeptide used in the vaccine comprises epitopes from Env and Gag. The polypeptide is conjugated to dextran and mixed with DNA, which leads to formation of microparticles presenting TBI on the surface and containing the DNA inside. The DNA (pcDNA-TCI) enclosed in the microparticles codes for the TCI (T cell immunogen) polypeptide, which contains $CD8^+$ and $CD4^+$ epitopes from Env, Gag, Pol, and Nef conserved among HIV subtypes A, B, and C.[1,2] The proposed technique enables the vaccine components to combine into particles on the principle of self-assembly (Fig. 1A).

The conjugate molecule consists of 1 dextran molecule, 1 protein TBI molecule, and 10–15 spermidine molecules. Positively charged spermidine provides binding of the conjugate dextran/protein TBI with negatively charged DNA-vaccine promoting formation of particles on the self-assembly principle.

According to our estimation, the plasmid pcDNA-TCI (6,583 bp) is able to present about 100–110 dextran (60 kDa) molecules on its surfaces. For vaccine assembly, pcDNA-TCI was added to conjugate in the proportion of 1 DNA molecule:120 conjugate molecules. To prove that CombiHIVvac has actually the form of particles, we used gel filtration chromatography and atomic force microscopy.[2] During sepharose CL-2B gel filtration, the vaccine was eluted in the volume, corresponding to 12–14 MDa size material.

Experimental visualization of a theoretically predicted formation of artificial microparticles was performed with transmission electron microscopy with negative staining (1%

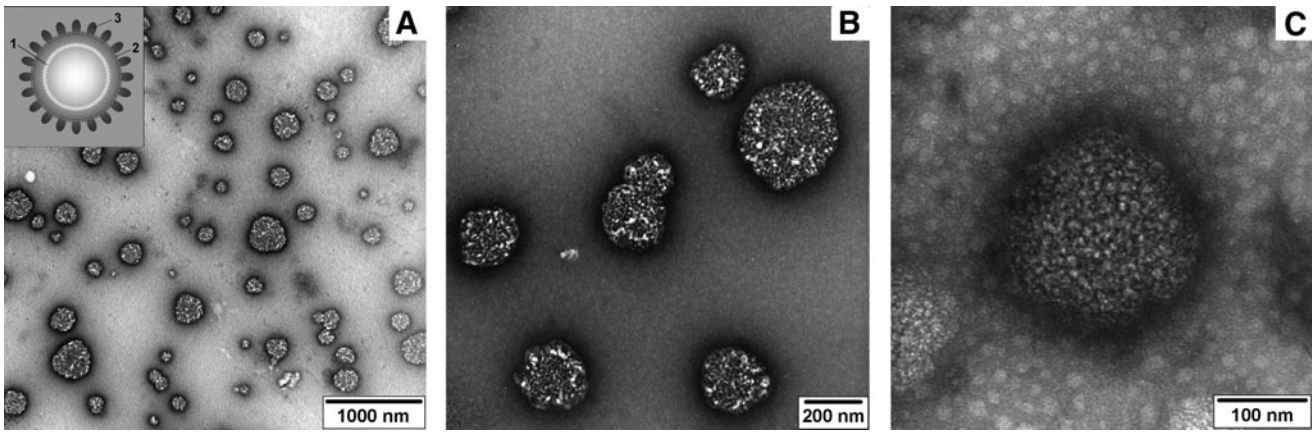

FIG. 1. Transmission electron microscopy images of CombiHIVvac microparticles with different magnification. **(A)** Scale bar 1,000 nm, the inset in the *left upper corner* is a scheme of a CombiHIVvac particle (1—pcDNA-TCI, 2—spermidine/dextran, 3—TBI); **(B)** Scale bar 200 nm; **(C)** Scale bar 100 nm.

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aqueous uranyl acetate solution). JEM 1400 electronic microscope (Jeol, Japan) with accelerating voltage 80 kV was used.

The obtained images demonstrate particles 50–250 nm in diameter (Fig. 1A–C). The morphological similarity of these objects to native virus particles is obvious.

Previously we showed that by combining two immunogens (TBI and TCI) in one construct, significant enhancement of HIV-specific B cell response was observed.[3] In our opinion, the formation of such particles plays a critical role in the registered effect. CombiHIVvac particles enable more effective absorption by antigen-presenting cells than individual molecules. As TBI protein is fixed on the particle surface and is represented in multiple copies, this provides multiple enhancement of vaccine antigenicity. Besides, pcDNA-TCI enclosed in the vaccine structure is more protected against degradation by DNase I than free pcDNA-TCI, as it was previously demonstrated,[4] resulting in prolongation of DNA-vaccine presence in organism. Finally, the presence of MHC class II restricted $CD4^+$ T-helper epitopes in the protein TCI may be the main reason underlying the increased synthesis of antibodies to TBI protein because of a CD4-mediated stimulation of B cell proliferation and differentiation.

Thus, the obtained high-resolution images of CombiHIVvac artificial microparticles show that vaccine structure mimics the size and organization of native viruses and yield insights into how this structure relates to high immunogenicity of the vaccine.

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Author Disclosure Statement

No competing financial interests exist.

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