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Structural Characterization of L1 Virus-Like Particles Used as Antigens in *Cervarix*™, the HPV-16 and HPV-18 Vaccine against Cervical Cancer

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Cervarix™ is a prophylactic human papillomavirus (HPV)-16 and -18 vaccine, developed for the prevention of cervical cancer. The vaccine antigens are HPV-16 and HPV-18 L1 virus-like particles (VLPs) made from recombinant HPV-16 and HPV-18 L1 proteins, respectively.

HPV-16 and HPV-18 L1 proteins are expressed at high levels in insect cells using the baculovirus expression vector system (BEVS). Under natural conditions, L1 migrates to the nucleus where virus assembly occurs. The L1 antigen used for the HPV-16/18 VLP vaccine is truncated at the C-terminus by 34 amino acids for HPV-16 and 35 amino acids for HPV-18. This truncation removes the nuclear targeting signal as well as the DNA binding domain. The distribution of HPV-16 and HPV-18 L1 in insect cells expressing the proteins was investigated by immunogold EM. Both HPV-16 and HPV-18 L1 proteins were found exclusively in the cytoplasm and no VLPs were detected. This confirms that the truncation prevented the migration of the L1 proteins into the nucleus and that VLP did not assemble intracellularly.

The L1 proteins were extracted and purified through a multistage process which, after self-assembly of L1 capsomeres, yields highly pure and immunogenic VLP. The physico-chemical and immunological properties of the HPV-16 and HPV-18 L1 VLPs have been thoroughly characterized [1], as well as their stability [2].

By negative staining EM, most VLPs appear single-shelled and subspherical in shape (Fig. 1). Their size, measured by disc centrifugation spectroscopy (DCS), was in good agreement with EM observations, ranging from 30 to 50 nm for HPV-16 L1 VLP, with a main peak at 40 nm. For HPV-18 VLP, the size ranged between 45 and 55 nm with a main peak at 50 nm. Both HPV-16 and HPV-18 L1 VLP populations had a small subset of multilayered VLPs with a size ranging from 60 to 75 nm. On average, the proportion of multilayered VLPs was 10.3 % and 7.8 % for HPV-16 and HPV-18 L1 VLP, respectively.

Protein Tomography™ (SIDEC Technologies) was applied to cryo-EM analysis of the VLP. It revealed at the surface of the VLPs a combination of pentameric and hexameric organizations of the capsomeres with a typical icosahedral symmetry (Fig. 2A). The 3D analysis also demonstrated that the structure of the large VLPs was either true concentric shells of capsomeres (2 or 3, rarely 4) or a compact spiral of capsomere ribbons. Protein Tomography™ also allowed visualizing the binding of monoclonal antibodies directed against conformational neutralizing VLP epitopes (Fig. 2B).

Finally, direct imaging by negative staining in TEM showed that VLPs adsorbed on aluminum hydroxide were indistinguishable from their counterpart in bulk solution (Fig. 3). It demonstrated that adsorption on aluminum hydroxide in formulated vaccine did not alter the structure of VLPs.

References

[1] M. Deschuyteneer et al., *Hum. Vaccin.* 6 (5) (2010), in press.
[2] D. Le Tallec et al., *Hum. Vaccin.* 5 (7) (2009) 467.
[3] We are grateful to Pascal Bourguignon for expert technical assistance with DCS and to Ulrike Krause for editorial assistance.

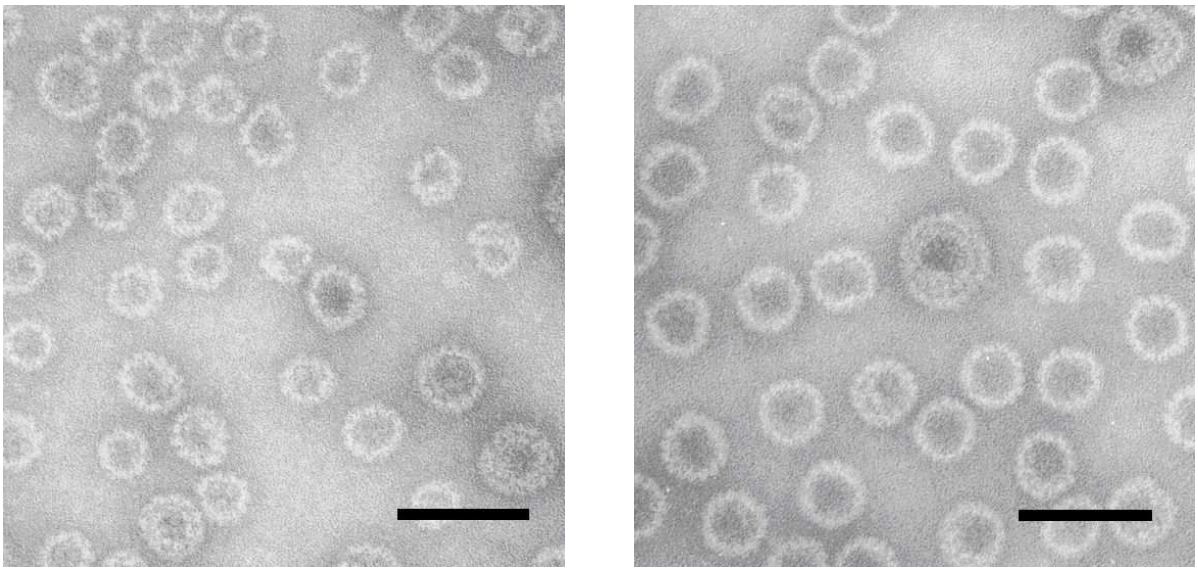

FIG. 1. HPV-16 (left) and HPV-18 (right) L1 VLP by negative staining. (Bar =100 nm).

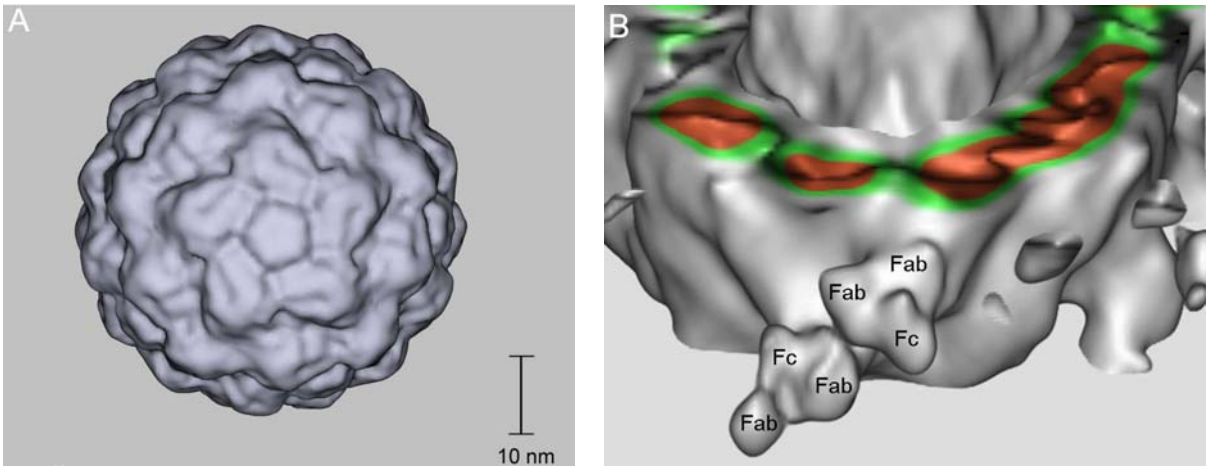

FIG. 2. A: Tomogram of a single HPV-16 L1 VLP (not averaging) (published in [1]).
B: Monoclonal antibodies bound to neutralizing conformational epitopes on a HPV18 L1-VLP.

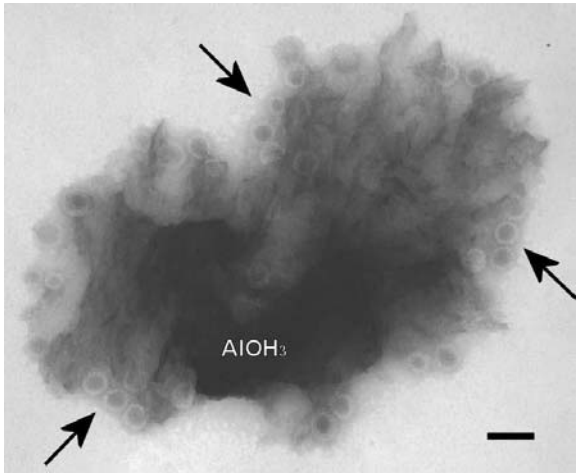

FIG. 3. VLP (arrows) adsorbed on aluminum hydroxide in the formulated vaccine (Bar = 100 nm) (published in [1]).

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