DEVELOPING PLANT-BASED CANDIDATE VACCINES AGAINST HIV-AIDS

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Introduction.
The third variable loop of the HIV gp120 protein contains neutralizing epitopes, thus is a relevant target for vaccine design (1). Efforts conducted by our research group on the development of plant-based candidate vaccines against HIV-AIDS will be presented.
The objective of this project was to develop a plant-based platform based on the expression of a V3-based multiepitopic protein in tobacco and lettuce plants.

Methods and Results. A multiepitopic protein named C4(V3)6 was designed in an effort to pursue broad immunization against the Human immunodeficienty virus (HIV). This C4(V3)6 chimeric protein is based on sequences of gp120, including epitopes from the fourth conserved domain (C4) and six tandem repeats of the third variable domain (V3), which represent different HIV isolates. The Histidine-tagged C4(V3)6 was subsequently over-expressed in a recombinant Escherichia coli strain, and purified by Immobilized Metal Ion Affinity Chromatography.
Tobacco and lettuce transgenic lines were developed by an Agrobacterium-based method (2). Leaves from mature plants were used to conduct expression analysis by Western blot and ELISA. Immunogenicity was assessed by intragastric administration of controls or lettuce expressing C4(V3)6 to Balb/c mice groups. Antibody responses were determined by ELISA against specific antigens.

Results. Expression of the C4(V3)6 in both tobacco and lettuce plants was achieved with no toxic effects on plant growth. The functional lettuce-derived C4(V3)6 protein showed HIV antigenic determinants and expression levels reached 240 µg/g DW in leaves (Fig. 1). Importantly, when orally administered to Balb/c mice, the lettuce-derived C4(V3)6 was capable of eliciting specific antibodies against pure C4(V3)6 (Fig. 2) and synthetic peptides containing distinct V3 sequences (data not shown).

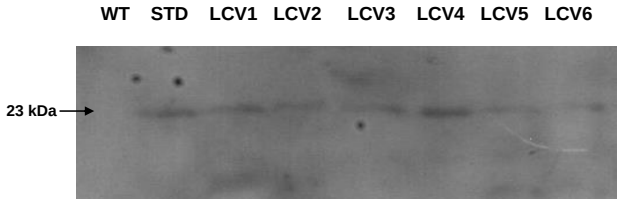

Fig. 1. Detection of the immunoreactive C4(V3)6 protein in lettuce extracts. Presence of the expected recombinant C4(V3)6 was determined by Western blot analysis. Lettuce-derived C4(V3)6 protein was detected by labeling with an anti-His tag antibody. Lines: WT, wild type lettuce; STD, 500 ng of pure C4(V3)6 as standard; and LCV1 to 6, transgenic lettuce lines.

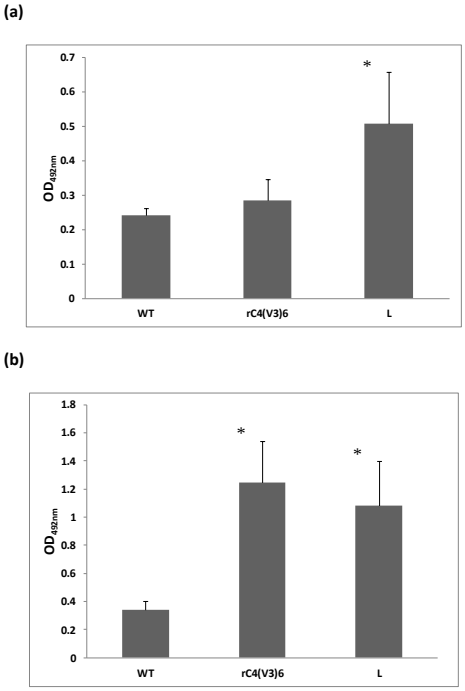

Fig. 2. Anti-C4(V3)6 IgA mucosal (a) and IgG serum (b) responses elicited in BALB/c mice. Test animal groups were dosed orally with wild-type lettuce material (WT), 100 µg of *E. coli*-derived C4(V3)6 (rC4(V3)6), or lettuce-derived C4(V3)6 (L). After dilution (mucosal 1:4; sera, 1:8), samples were analyzed by ELISA. Mean OD_{492} nm values ± SD from each experimental group (n = 5, analyzed by triplicate) are shown. *P < 0.05 vs. the WT group.

Conclusions. The protein C4(V3)6 is successfully expressed in lettuce retaining its immunogenic properties when orally administered, which point out this multiepitopic protein as candidate for further preclinical evaluations.

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References.

1. Curtis IS (2006) Lettuce (*Lactuca sativa L.*). *Methods Mol Biol* 343:449-458
2. Daniell H, StreatWeld SJ, WycoV K (2001) Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci* 6:219-226
3. Varona-Santos JT, Vázquez-Padrón R, Moreno-Fierros L (2006) Production of a short recombinant C4V3 HIV-1 Immunogen that induces strong anti-HIV responses by systemic and mucosal routes without the need of adjuvants. *Viral Immunol* 19:237-249.