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Poster Abstract – F.10

THE DEVELOPMENT OF TRANSGENIC AND TRANSPLASTOMIC PLANTS FOR PRODUCTION OF A TUBERCULOSIS SUBUNIT VACCINE

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Mucosal delivery of a vaccine can induce systemic and mucosal immune responses and eliminate the need for needle and syringes. Transgenic plants are an ideal means to produce mucosal vaccines, as the plant cell wall protects the antigenic proteins from the acidic environment of the stomach. The purpose of this study was to evaluate the potential of an oral, plant-made, tuberculosis vaccine based on the immunodominant antigen ESAT-6 (6-kDa early secretory antigenic target). A synthetic, plant-optimized, coding region was constructed for the antigen ESAT-6. The resulting coding region was fused to the B subunit of the *Escherichia coli* heat-labile enterotoxin (LTB) to promote targeting to the antigen presenting cells beneath the lining of mucosal surfaces. The gene for the LTB/ESAT-6 fusion protein has been expressed in the model plant species *Arabidopsis thaliana* and in *Lycopersicum esculetum* (tomato). Both *A. thaliana* and tomato produced a fully assembled and functional antigen. Preliminary results indicated that, in mice, oral delivery of the plant-made LTB-ESAT-6 fusion protein induced antigen-specific responses from $CD4^+$ cells and IFN-g. In addition, a type 2 response was induced in the Peyer's Patch. Thus, the plant-made antigen was delivered to the gut-associated lymphoid tissue.

In comparison with conventional nuclear transformation, plastid transformation is reported to have significant advantages, such as high gene expression and transgene containment. Hence, in this study, the fusion gene LTB/ESAT-6 was cloned also in plasmids for stable plastid integration and expression. Transplastomic tobacco plants were produced by bombardment of tobacco leaves with the construct containing the fusion gene LTB/ESAT-6. Incorporation of the fusion gene in the plastid genome was confirmed by polymerase chain reaction (PCR) analysis. Western Blot and ELISA analyses are under way to verify transgene expression, product size and estimate antigen expression level.