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EXPRESSION OF A MUTATED FORM OF hGAD65 IN TRANSGENIC TOBACCO PLANTS

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Type 1 insulin-dependent diabetes mellitus (T1DM) which afflicts 0.2-0.3% of population is caused by autoimmune destruction of insulin-secreting beta cells. The young age of affected patients, the need for life-long insulin therapy and the high prevalence of late-onset complications make T1DM a major health problem. The smaller isoform of glutamic acid decarboxylase of 65 kDa (GAD65) is a major autoantigen in human T1DM. Induction of oral tolerance has been reported to modify the natural history of several autoimmune diseases both in experimental models and in pilot human trials. Studies in animal models of spontaneous autoimmune diabetes have shown that parenteral administration of GAD65 can prevent (or delay) the onset of disease.

Poor GAD protein solubility in bacteria and inadequate production from eukaryotic cells have so far precluded the use of this approach for the large scale production of GAD65 for oral tolerance studies.

Transgenic plants that express high level of recombinant human GAD65 could be the source of food for oral administration of the autoantigen. We previously reported the production and characterization of transgenic plants expressing membrane-anchored hGAD65 (Porceddu *et al.,* 1999) and the production and characterization of plants expressing a cytosolic form of the recombinant protein (GAD67/65) (Avesani *et al.,* 2003). By using a radio-immuno assay with human serum from a GAD65 autoantibody positive T1DM patient, the highest expression level of the recombinant GAD67/65 protein was estimated to be 0.19% of total soluble protein, compared to only 0.04% of hGAD65.

In a recent study we tested the hypothesis that the expression level of recombinant hGAD65 could be improved by using a mutated form of the enzyme with no catalytic activity (hGAD65mut).

We transformed Nicotiana tabacum oklants with seven constructs for the expression of the protein in different sub-cellular compartments.

Tobacco transformed plant were characterised by western blot, radio immuno-assay analysis and real time RT-PCR. The use of the mutated form of the enzyme causes the improvement of GAD expression in transgenic plants.

The constructs used and the relative expression data are discussed.