

**Poster Abstract – F.01**

---

**THE SEEDS OF HOPE**

F. MORANDINI\*, L. AVESANI\*, M. BRUSCHETTA\*, B. VAN DROOGENBROECK\*\*,  
A. DEPICKER\*\*, A. FALORNI\*\*\*, M. PEZZOTTI\*

\*) Department Scientific and Technological, School of Biotechnology , University of Verona,  
Strada Le Grazie 15, 37134 Verona, Italy

\*\*) VIB2 – Department of Plant Systems Biology, Ghent University, Technologiepark 927, B-9052  
Ghent, Belgium

\*\*\*) Department of Internal Medicine, University of Perugia, Via Dal Pozzo, 06126 Perugia, Italy

*autoimmunity, diabetes mellitus, GAD65, oral tolerance, transgenic plants*

Type 1 insulin-dependent diabetes mellitus (T1DM) is caused by autoimmune destruction of insulin-secreting  $\beta$  cells and afflicts 0.2-0.3% of the population. The young age of affected patients, the need for life-long insulin therapy and the high prevalence of late-onset complications make this disease an enormous 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 (NOD mouse) have shown that parenteral and nasal administration of GAD65 can prevent (or delay) the onset of the disease. Induction of oral tolerance requires a prolonged administration of autoantigens, in the range of mg/week/mouse.

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 expressing high level of recombinant human GAD65 could be a new and economic source of food for oral administration of the autoantigen. We previously reported the production and characterization of transgenic plants that express 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 (RIA) 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.

To improve expression levels of recombinant hGAD65 we have used seed-specific expression signals for seed accumulation in different plant species. The sequence encoding GAD67/65 enzymatically inactive (mutated in the active site) was cloned between the N-terminal signal sequence of the seed storage protein 2S2 for ER-targeting and the C-terminal KDEL sequence for ER-retention. This open reading frame was brought under control of the  $\beta$ -phaseolin promoter of *P. vulgaris* with an  $\Omega$  leader and of the 3' regulatory sequence of the arc5-I genomic clone. This expression cassette was used to transform *N. tabacum* and *A. thaliana* plants. To compare different plant expression systems we also plan to transform *P. hybrida* and *M. truncatula* plants.

Total soluble proteins were extracted from seeds of transgenic tobacco and Arabidopsis plants and Western blot analysis was performed to test the presence of the recombinant hGAD65. The highest expression level of hGAD65 was estimated to be 0.1% of the total soluble proteins.