Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 5C.17

EXPRESSION OF N-TRUNCATED GAD65mut FORMS IN A PLANT-BASED PLATFORM

MERLIN M.*, GECCHELE E.*, BROZZETTI A.**, AVESANI L.*, PEZZOTTI M.*

*) Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona (Italy)
**) Department of Internal Medicine, University of Perugia, Via dal Pozzo, 06126 Perugia (Italy)

T1D, GAD65, Nicotiana benthamiana, transient expression

Type 1 diabetes (T1D) is an autoimmune disease characterized by the T-cell mediated destruction of insulin-secreting pancreatic β-cells, causing the need of life-long insulin therapy. The 65 kDa isoform of glutamic acid decarboxylase (GAD65) present in the pancreatic cells is one of the major autoantigen involved in disease development. In the last years antigen-specific immunotherapy (ASI) based on the delivery of GAD65 has emerged as an appealing approach for treating T1D; recent phase II clinical trials have shown that human administration of two injections of 20 μg of alum-formulated GAD65 lead to a significant preservation of residual insulin secretion without serious adverse effects. Large-scale phase III confirmatory studies are underway in Europe and in the USA. The major disadvantage of this approach is the high-cost associated with the current molecule production system based on Baculovirus/insect cells (500,000 €/g). In the perspective of the use of GAD65 for autoimmune treatment a cost-effective recombinant system for the production of the immunoreactive protein would be highly desirable.

It is well documented that GAD65 undergoes some post-translational modifications in the N-terminal domain that result in a firmly membrane-anchored protein, which is highly hydrophobic. In vitro, GAD65 requires detergent to be solubilised. However, because detergents are extremely cytotoxic, a detergent-free preparation is mandatory for vaccination; moreover the presence of detergents can complicate the purification process. The production of a soluble form of the protein would simplify the downstream processing of the molecule and, eventually, the final pharmaceutical formulation.

We have previously shown that GAD65 and a mutated catalytically-inactive form of the protein (GAD65mut) can be expressed in transgenic tobacco plants. GAD65mut accumulates 10-fold higher than GAD65 and retains the immunogenic properties.

In order to develop a system for the high-efficient production and purification of GAD65, we engineered GAD65mut to various extents to obtain soluble forms of the molecule. In the present work we describe and discuss the solubility and accumulation levels of three N-truncated forms of GAD65mut in comparison with full-length GAD65mut and GAD65 in a plant-based platform. This system, based on transient expression in *N. benthamiana*, was chosen for its high-throughput and fast expression of molecules.