EXPRESSION OF A MUTATED FORM OF GAD65 IN HETEROLOGOUS SYSTEMS


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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 the major autoantigen in human T1DM and it has recently demonstrated that two injections of the molecule can give protection against this autoimmune disease.

T1DM requires a primary prevention because the disease has a complex genetic basis, making difficult to identify in the population people at risk of developing it. Vaccination studies and subsequent vaccination treatment of a lot of people need large quantity of purified protein, but the current production systems are too much expensive and unable to provide enough GAD65 to meet global demand.

We have previously shown that GAD65 can be expressed in transgenic tobacco plants but yields are disappointing. In order to improve its expression level we use different heterologous systems such as Nicotiana tabacum plants, E.coli inducible system and insect cells/Baculovirus to express two different forms of the recombinant human GAD65: the wild type form of the enzyme (hGAD65) and the mutated form with no catalytic activity (hGAD65mut), hypothesising that the enzymatic activity might interfere with its accumulation in heterologous systems.

In previous studies it has been demonstrated in vitro the lack of the enzymatic activity for the hGAD65mut and we show that GAD65mut accumulates to higher levels in transgenic plants and in E.coli inducible system than its enzymatically active counterpart, indicating that the catalytic properties of GAD65 contribute to its poor yields.

To demonstrated the absence of enzymatic activity of the mutated form of GAD65 (GAD65mut) also in the heterologous systems we perform an enzymatic assay in vivo., The results of the assay and the difference among the expression levels obtained in the heterologous systems are discussed.