Detection kit for autoantibodies against enteric alpha-defensins

Human enteric alpha-defensin HD5 has been recently identified by our laboratory as a novel and clinically relevant autoantigen in autoimmune disease called APECED (Dobes et al, 2015, Gastroenterology 149:139-50). Since up to 30% of APECED patients are seropositive for autoantibody to HD5, its detection could serve as a valuable marker for ongoing intestine autoimmune process. In order to provide a tool for experimental and clinical detection of serum anti-HD5 autoantibody, we cloned, expressed and tested recombinant HD5 (rHD5) for its specificity and sensitivity towards serum isolated from anti-HD5 seropositive patients. HD5 gene was amplified and cloned into TrpLE(HT)pAED4 vector (Fig. 1) and its product was expressed, isolated and detected by Coomasie stained gel (Fig. 2). Subsequent experiments confirmed that rHD5 can be very sensitively recognized by a commercially available antibody at high dilution rate (Fig. 3), as well as by defensin-seropositive sera from APECED patients (Fig. 4 and 5) but not by a serum from a healthy control (Fig. 4).

Fig.1. Schematic representation of the expression vector pAED with inserted sequences coding for a portion of the tryptophan operon of E. coli (trp ΔLE 1413) and the (His)6 affinity-tag (labeled “Trp(HT)”). P (located between the HindIII and BamHI sites) indicates the position of the DNA inserts coding for the mature α-defensins. (M Pazgier, J Lubkowski, Expression and purification of recombinant human α-defensins in Escherichia coli, Protein Expr Purif. 2006 Sep;49(1):1-8.)

Fig.2. Coomasie blue gel staining of DEFA5 protein isolated from E.coli after [Isopropylβ-D-1-thiogalactopyranoside (IPTG)] induction. Def1+ to Def9+ represents the first to ninth fraction of TrpLE(HT)pAED4-DefA5 during releasing protein from inclusion bodies. Def1- to Def9- represents the empty vector TrpLE. Def9+ (10x) represents the final TrpLE(HT)pAED4-DefA5 isolation and was loaded in 10x higher concentration compared to other fractions.
**Conclusions:** The results show that newly prepared recombinant human enteric defensin HD5 (rHD5) is a suitable substrate to detect the presence of autoantibodies in patients suffering from APECED or potentially also from other types of intestine-related autoimmunity. The construction of ELISA HDS-specific autoantibody detection kit is currently under construction.

To get more information about the rHD5 or to buy nonexclusive licence for its production, please contact Center for Technology Trasfer, IMG AS CR, Videnska 1083, 14220 Praha 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).