

## Calreticulin – an autoantigen in autoimmune and tumor diseases

**Introduction:** Calreticulin, a calcium binding protein and chaperon, is a common autoantigen in autoimmune diseases. Antibodies (Ab) against calreticulin (CRT) were recently detected in sera of some patients with hepatocellular carcinoma (CaH) and pancreatic tumors (CaP) using a combination of two-dimensional electrophoresis and Western blotting. **Aims:** The aims of this study were a) to quantify the levels of IgA and IgG Ab against CRT in sera of patients with CaH, CaP (metastatic, non metastatic), colorectal carcinoma, pancreatitis and viral hepatitis. b) to analyse the antigenic epitopes of CRT, c) to prepare the first half of human recombinant CRT. **Methods:** Direct ELISA test with human recombinant CRT as antigen was used for testing the levels of serum Ab. Antigenic epitopes of CRT recognised by serum IgA and IgG Ab were analysed by PEPSCAN method in patients with CaH and CaP. The first half of human recombinant CRT was prepared by recombinant technology and subsequently purified by affinity chromatography. **Results:** We found significantly elevated levels of IgA anti-CRT Ab in sera of patients with CaH ( $P < 0,01$ , 8/13), CaP ( $P < 0,05$ , 17/38), CaP with liver metastasis ( $P < 0,01$ , 2/8) and pancreatitis ( $P < 0,05$ , 4/14) in contrast to healthy controls (2/35). Significantly elevated levels of IgG anti-CRT Ab were detected in sera of patients with CaH ( $P < 0,01$ , 8/13) and CaP ( $P < 0,01$ , 15/38) in contrast to healthy controls (2/35); b) IgA Ab of all patients with CaH and CaP reacted with amino acid sequences EVKIDNSQVESG and RKEEEEAEDKED on CRT molecule. IgG Ab of patients with CaH and CaP bound the same amino acid sequence EEDKKRKEEEEAEDKED and with lower frequency also the peptides FAEAPFSNKGQT, ERAKIDDPTD and DKAPEHIPDP, c) The first half of human recombinant CRT was associated with inclusion bodies after its cell expression; for this reason we solubilised this fragment by urea and subsequently purified it under denaturising conditions. **Conclusions:** Our results suggest the existence of epitopes specifically recognised by Ab of both patients with CaH and CaP. Testing of the Ab specifically binding the epitopes could be useful for diagnostic or prognostic purposes.