

## ABSTRACT

Distinct cellular level of the  $\text{Ca}^{2+}$  binding chaperone calreticulin (CRT) is essential for cardiac development and postnatal function. However, CRT is also a potential autoantigen eliciting formation of antibodies (Ab), whose role is not yet clarified. Immunization with CRT leads to cardiac injury, and overexpression of CRT in cardiomyocytes induces dilated cardiomyopathy (DCM) in experimental animals. Hence, we analysed levels of anti-CRT Ab and calreticulin in the sera of patients with idiopathic DCM and hypertrophic cardiomyopathy (HCM). ELISA and immunoblot using human recombinant CRT and Pepscan with synthetic, overlapping decapeptides of CRT were used to detect anti-CRT Ab. Significantly increased levels of anti-CRT Ab of IgA ( $P < 0.001$ ) and IgG ( $P < 0.05$ ) isotypes were found in patients with both DCM (12/34 seropositive for IgA, 7/34 for IgG) and HCM (13/38 seropositive for IgA, 11/38 for IgG) when compared with controls (2/79 for IgA, 1/79 for IgG). Titration analysis in seropositive DCM and HCM patients documented anti-CRT Ab detected at 1/1600 dilution for IgG and 1/800 for IgA (and IgA1) and at least at 1/200 dilution for IgA2, IgG1, IgG2 and IgG3. Pepscan identified several immunogenic CRT epitopes: EVKIDNSQVESGSLED, IDDP TDSKPE, DKAPEHIPDPDA and RKEEEEAEDKEDDAEDKDEDEEDE recognised by IgA and IgG Ab of these patients. Serum CRT concentration was tested by ELISA. Significantly increased levels of CRT relative to healthy controls were found in sera of patients with HCM ( $P < 0.01$ , 5/19). Six out of 17 patients with DCM and 1 out of 24 healthy controls were seropositive for serum CRT. These data extend the knowledge of seroprevalence of anti-CRT Ab and CRT, and suggest possible involvement of autoimmune mechanism in some forms of clinically heterogeneous cardiomyopathies.

**Key words:** calreticulin, anti-calreticulin antibodies, autoimmunity, antigenic epitopes, dilated cardiomyopathy, hypertrophic cardiomyopathy, ELISA, Pepscan