

## Clinical anatomic, immunomorphologic and molecular anatomic data suggest interplay of thyroidal molecules, autoantibodies and Hsp60 in Hashimoto's disease

Antonella Marino Gammazza,<sup>1,2</sup> Manfredi Rizzo,<sup>2,3</sup> Roberto Citarrella,<sup>3</sup> Francesca Rappa,<sup>1,2</sup> Claudia Campanella,<sup>1,2</sup> Fabio Bucchieri,<sup>1,2</sup> Angelo Patti,<sup>2,3</sup> Dragana Nikolic,<sup>1,3</sup> Daniela Cabibi,<sup>4</sup> Giandomenico Amico,<sup>5</sup> Pier Giulio Conaldi,<sup>5</sup> Pier Luigi San Biagio,<sup>6</sup> Giovanni Peri,<sup>1</sup> Giuseppe Montalto,<sup>3</sup> Felicia Farina,<sup>1</sup> Giovanni Zummo,<sup>1</sup> Everly Conway de Macario,<sup>7</sup> Alberto JL Macario<sup>2,7</sup> and Francesco Cappello<sup>1,2,6</sup>

<sup>1</sup> Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Palermo, Italy

<sup>2</sup> Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy

<sup>3</sup> Department of Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy

<sup>4</sup> Department of Human Pathology, University of Palermo, Palermo, Italy

<sup>5</sup> ISMETT – Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione and RIMED Foundation, Palermo, Italy

<sup>6</sup> Institute of Biophysics (IBF), National Research Council of Italy (CNR), Palermo, Italy

<sup>7</sup> Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore, and IMET, Columbus Center, Baltimore, MD, USA

Hsp60 is, typically, a mitochondrial protein, but it also occurs in the cytosol, vesicles, and plasma membrane, and in the intercellular space and biological fluids, e.g., blood. Changes in the levels and distribution of Hsp60 are linked to several pathologies, including cancer and chronic inflammatory and autoimmune disorders. What is the histopathological pattern of Hsp60 in the thyroid of Hashimoto's patients? Are there indications of a pathogenic role of Hsp60 that may make Hashimoto's thyroiditis a chaperonopathy? Experiments reported here provide information regarding those questions. We found by various immunomorphological techniques increased levels of Hsp60 in the thyroid from HT patients, localized to thyrocytes of small and degenerated follicles and to oncocytes (Hurtle cells). Immunofluorescence showed the chaperonin both inside the cells and also in the plasma membrane, especially in oncocytes. We also found that Hsp60 levels in the blood of HT patients were increased compared to controls and correlated with those of autoantibodies against two distinctive thyroidal proteins, thyroglobulin (TG) and thyroid peroxidase (TPO) ( $r=0.379$ ,  $p=0.0103$ ;  $r=0.484$ ,  $p=0.0008$ ; respectively). Molecular analysis of these two proteins in comparison with Hsp60 demonstrated various regions of high structural similarity shared by them, which could very well be immunologically crossreactive epitopes. Thus, it is likely that the three proteins potentiate each other as immunogens to elicit autoantibodies and, as antigens, to cause antigen-antibody reactions at those sites in which Hsp60 is exposed, for example the surface of oncocytes. This would lead to inflammation and oncoyte lysis with destruction of thyroidal tissue. The cytometric bead assay revealed that recombinant Hsp60 did not induce increment of cytokine production by peripheral blood mononuclear cells from HT patients. Consequently, we propose that Hsp60 is implicated in the pathogenesis of Hashimoto's thyroiditis as autoantigen, via a participation of autoantibodies that also recognize TG and TPO, whereas participation of inflammatory cytokines induced by the chaperonin is unlikely. Supported by IEMEST (FC and AJLM).