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HLA- DR3, DQ2 transgenic mice for identification of immunodominant peptides of pancreatic autoantigens

Background and Aims: Sardinians have one of the highest insulin dependent autoimmune diabetes mellitus (T1DM) incidence in the world (38/100.000 in the age range 0-14 years) associated with a low grade of genetic heterogeneity and a high frequency of the HLA- DR3-DQ2 haplotype. This HLA haplotype confers a high risk for T1D, celiac disease, and multiple sclerosis. The aims of the study are: a) the identification of immunodominat T cell epitopes of preproinsulin and GAD65 pancreatic autoantigens immunizing HLA- DR3-DQ2 transgenic mice with these autoantigens and deriving T cell specific hybridomas and b) the design of variant peptides of these epitopes able to bind HLA class II molecules without triggering T cell activation.

Methods: HLA- DR3-DQ2, human CD4, IA class II KO triple transgenic mice in the NOD background will be immunized with human PPI and GAD65 autoantigens to generate antigen specific T cells. These cells will be fused with the BW5147 cell line to produce T cell hybridomas and specific T cell responses will be identified stimulating the hybridomas with overlapping peptides of PPI and GAD65 and measuring IL-2 production. PPI and GAD65 will be produced in vitro, purified and quantified in order to immunize each mouse with 100 µg of antigen. The pepscan of overlapping PPI and GAD65 13 Mer peptides will be purchased from the Alphalyse Company.

Results: With the aim of improving T cell response and the generation of antigen specific T cell hybridoma, the HLA-DR3-DQ2 transgenic mice, initially obtained in the C57BL/6, have been backcrossed in the class II KO human CD4 NOD mice. These animals are more suitable for immunization since they maintain the autoimmunity background of NOD mice (an animal model of spontaneous human T1DM) in absence of murine class II molecules. At the present time these animals have obtained and are maintained in our animal facility. Moreover, the expression vector to produce both PPI and GAD65 has been generated.

Conclusions: With the present study the portion more immunogenic of PPI and GAD65 pancreatic autoantigens presented by HLA, DR3 and HLA DQ2 molecules will be identified. This information will be used to construct variant peptides able to bind diabetogenic HLA class II molecules without triggering a T cell activation providing a possible peptide vaccine for T1DM.

Patel SD, Cope AP, Congia M, Chen TT, Kim E, Fugger L, et al. Identification of immunodominant T cell epitopes of human glutamic acid decarboxylase 65 by using HLA-DR(alpha1*0101,beta1*0401) transgenic mice. Proc Natl Acad Sci U S A. 1997;94:8082-7.

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Biography

Sandro Muntoni completed his degree in Medicine 1990 at Cagliari University Medical School. From 1991-1992 he is a research fellow at the department of Clinical Immunology at the London Hospital Medical College, London, UK. He is an Associated Professor of Pathology, Toxicology Department, Unit of Oncology and Molecular Pathology at University of Cagliari Medical School, Cagliari, Italy. He is a Member of the National Council of Scientific Societies for the reduction of Cardiovascular Risk and he is an author of over 75 scientific papers.

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