

ImmunoTools *special* Award 2013



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Study of the presence of soluble molecules in the serum of patients with autoimmune diseases modulating the immune responses.

The research proposed in the present application intends to contribute in the knowledge of the genetic background and of the immunopathological mechanisms of Primary biliary cirrhosis (PBC) by studying selected, functionally relevant polymorphic genes, such as *CTLA-4*. The results will be analyzed taking into account autoantibodies status, type of clinical presentation and possible association with other autoimmune diseases.

Primary biliary cirrhosis (PBC) is a chronic autoimmune cholestatic liver disease frequently characterized by anti-mitochondrial autoantibodies (AMA). A minority of patients are AMA-negative. Cytotoxic-T-Lymphocyte-Antigen-4 (CTLA-4) is a surface molecule expressed on activated T-cells delivering a critical negative immunoregulatory signal.

A soluble form of CTLA-4 (sCTLA-4) has been detected at high concentrations in several autoimmune diseases, and its possible functional meaning has been suggested.

The aim is to evaluate the presence of sCTLA-4 concentration in sera of patients with PBC and to correlate it to immunological abnormalities associated with the disease.

The ultimate objectives are:

- defining additional genetic markers for PBC and determining their possible usefulness in discovering high-risk individuals;
- analysing their possible functional role in regulating the immune response;
- defining their possible relationship with subgroups of PBC patients (different clinical presentation) or their association with other autoimmune diseases.

Key points of the project are:

- *Evaluation of the presence of soluble form of CTLA-4 in serum of CD patients*

In this task we will analyze the presence of sCTLA-4 in the serum of CD patients (about 200) in comparison to that obtained from a group of healthy donors. This screening allows us to define two groups of PBC patients: positive and negative for sCTLA-4.

- *Functional role of CTLA-4*

The analysis of the possible functional role of sCTLA-4 present in PBC patient sera will be performed as follows described:

- *Mixed leukocyte reaction (MLR)*.

Two-way MLR will be used to test the immunoregulatory activity of sCTLA-4. The cells used in these experiments were obtained from a panel of previously HLA typed laboratory volunteers and are selected to provide two HLA-DR mismatches. The proliferation tests will be performed following the standard procedures. Sera from at least 20 PBC patients (selected on their sCTLA-4 levels) and from 10 normal donors will be added to the test.

An analogous test of cytokines production will be carried out using the PBMC of the CD patients so as to put in evidence possible different behaviour in the proliferation and cytokines pattern of these cells in presence of sCTLA-4. Moreover, we will estimate the phenotype of the peripheral T lymphocytes before (resting) and at different time during the phase of activation. The comparison of these results with T lymphocytes obtained from healthy donors will allow us to show the possible differences in the behaviour of T cells in response to the activatory stimulus in presence or absence of soluble CTLA-4. The minimal sample to analyse in this phase is constituted from 10 patients (subdivided in positive and negative for autoantibodies) and 10 negative controls. Cytokines will be measured by ELISA kits purchased from **ImmunoTools**.

ImmunoTools special AWARD for **Daniel Saverino** includes 20 reagents

PE - conjugated anti-human Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V, recombinant human cytokines: rh CTLA-4 / CD152

human IL-4 ELISA-set, human IL-6 ELISA-set, human IL-8 ELISA-set, human IL-12p40 ELISA-set, human TNF-alpha ELISA-set, (the ELISA sets contains each 3 reagents)

[DETAILS](#)