ImmunoTools *special* Award 2018

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Targeted peptide therapy in autoinflammatory diseases

Background: Autoimmunity is a failure of the body's immune system to recognize its own cells and tissues as "self", leading in certain cases to serious diseases and/or to deleterious functional changes in vital organs. Systemic lupus erythematosus (SLE) is the prototype of systemic autoimmune diseases. It is a chronic, relapsing-remitting autoinflammatory syndrome manifested with multiple and heterogeneous symptoms. It primarily affects women of reproductive age and its frequency is elevated in black and Asian females. Its aetiology, which appears multigenic, is not fully understood. Intense research is focused both on its origin and on the possible triggering factors that exacerbate the disease (environment, hormones, infection).

Nowadays there is no specific treatment for SLE. Efficient drugs that are currently given to patients often generate unwanted side effects. Thus, deciphering the immune circuits that are altered in lupus could disclose many possible targets for developing selective therapeutics. A number of trials have been conducted so far, however, many gave mitigate results only. New products that emerge from recent investigations are based on T cells (TNF-α receptor antagonists, IFN-γ signalling inhibitors) and B cells targeted and non-specific immunosuppressants, which block the signals that overstimulate immune responses.

Peptides are small, soluble molecules, which may prove to be very efficient at modulating the patterns of autoreactivity. The past 25 years of research from our lab led us to develop a 21-mer peptide called P140 (derived from the spliceosomal U1-70K protein), which display valuable properties to rescue individuals from lupus disease, both in model mice and patients. My team has shown that P140 acts via autophagy and more precisely chaperone-mediated autophagy, which is overactivated in lupus setting. The peptide decreases the over-expression of MHC class II molecules at the surface of antigen-presenting B cells. Although some autophagy-related genes might be involved, in fact, the origin of autophagy dysfunctioning is not known

Objective: To examine whether autoantibodies (autoAbs) to autophagy components exist in lupus and if they are pathogenic in humanized murine models.

Methodology: Having found that autoAbs to autophagy process elements effectively exist in the serum of autoimmune individuals, in the ongoing study, we want to test the pathogenicity of these autoAbs, which we purify from the patient's sera. This requires a large number of costly analyses, purification of human autoAbs by affinity chromatography, injection into recipient animals and finally studies of many markers on cell and tissues from recipients (humanized and naive mouse models). We want to test the autoAbs from ethnically distinct populations (European, Asians, and Americans) and determine the direct/indirect role of these Abs in the pathogenesis of SLE (unrelated Abs will be used as a control). All these experiments will be done with authorization for ethics and comfort of mice (already acquired).

- Human serum and characterization of autoAbs by conventional ELISA method.
- Cytokine estimation in sera.
    - Determine the serum levels of major pro- and anti-inflammatory cytokines (IFN-α, TNF-α, IL-12, IL-6, IL-1β, IL-10, IL-17) will be measured using ELISA and flow cytometry methods.
    - In parallel, peripheral blood mononuclear cells will be isolated from healthy controls and SLE patients and cultured for 24-48 h with increasing concentrations of affinity-purified high titer Abs derived from SLE patients. Levels of pro- and anti-inflammatory cytokines will be measured in culture supernatants.
- Evaluation of serum reactivity (lupus serum Abs to dsDNA, chromatin, nucleolin, HSP90, Hb, and others).
    - Animal experiments, read-outs: Body weight variation, lupus clinical symptoms (biochemical and behavioural), cytokine evaluation (*in vivo* and *ex vivo*), histology of major organs, immunophenotyping (CD3, CD4, CD8, CD19 cells, and other cells subsets, with co-stimulatory molecules CD28, CD80/86 and CD40/CD40L), and measurement of autophagy flux variation, determination of CMA activity

Expected outcome: These studies should provide unique information on the pathogenicity of autoAbs from different populations and help us to acquire a knowledge of novel therapeutic strategies for progressive SLE.

Reagents: **ImmunoTools** reagents helpful in conducting the above-mentioned studies.

ImmunoTools *special* AWARD for **Srinivasa Reddy Bonam** includes 25 reagents

FITC - conjugated anti-mouse	CD3e, CD4, CD8a, CD11b, CD45, CD45R, CD54, CD62L, CD80, NK-cells, αβ TCR, γδ TCR.
PE - conjugated anti-mouse	CD19

mouse ELISA-set (for one 96 plate): mouse IL-6, mouse IL-17A, mouse TNF-alpha

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