

Nuclease Abzymes Activity Assessment using Different DNA Substrates in Patients with Early Rheumatoid Arthritis

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Abstract: Background Autoimmune mechanisms of RA are regarded as a certain immune regulation disbalance with high production of autoantibodies. The main goal of our study was to assess putative nuclease activity of RA IgG towards different DNA substrates.

Objectives IgG samples of patients with early and healthy persons were examined. All patients were fulfilled 2010 Rheumatoid Arthritis Classification Criteria.

Materials and methods IgG were purified from the sera by combined method of affinity chromatography on protein A column. For the assessment of nuclease IgG activity it has been used plasmid dsDNA (DIARECT AG), DNA of *Cl. trachomatis* and PCR products: aggR (100 bp) and stx2e (733bp) as substrate of reaction. The reaction mixture (0.3 ml) contained 0.1 ml DNA substrate (100 µg/ml), 0.1 ml of IgG samples (1 mg/ml) or 0.1 ml of 0.9% NaCl for control sample, and 0.1 ml 0.02 M Tris-HCl (pH 7.4) with 0.01 M MgCl₂. It was incubated for 20 hrs at 37°C. DNase activity was registered with agarose gel electrophoresis. The reaction mixture (10 µl) was subjected to electrophoresis in 1,5% agarose (Fluka, BioChemika,) in Tris-borate buffer (89 mM Tris base, 2.5 mM disodium EDTA, and 8.9 mM boric acid) with ethidium bromide (0.4 µg/ml). Electrophoresis was carried out in a horizontal slab gel apparatus. The dimensions of the gel were 8.6 by 12.7 by 0.6 sm and 10 by 10 by 0.5 sm. Sample wells were made by use of a polystyrene comb with 14 teeth, each 0.3 cm wide and spaced by 0.6 cm. Electrophoresis was carried out at 30 mA, 120 V, for 2 h or until the dye neared the bottom of the gel.

Results It has been shown that early RA patients demonstrated different patterns of DNA degradation upon abzyme action. Some patients samples rendered full DNA hydrolysis at 20 hours incubation, whereas others samples displayed parcicularly ability to degaradate DNA. We obseved that abzymes hydrolyze long DNA substrate (700 bp and longer) in comparison with short one (100 bp).

Conclusions For the first time we confirmed the presence abzyme IgG activity against different DNA substrate in patients with early RA. The different patterns of DNA degradation upon abzyme action were confirmed. Futher it seems worthy to study other DNA substrates to assign various kinds of DNase IgG activity to certain subsets of RA.

Key words: abzymes, DNA, rheumatoid arthritis