

European Journal of Immunology

Supplement
1/09

**Immunity for Life
Immunology for Health**

Abstracts

2nd European Congress of Immunology

September 13–16, 2009

Berlin, Germany

www.eci-berlin2009.com

www.eji-journal.eu



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Because PRLr stimulate the expression of a variety of genes that are involved in cell proliferation and differentiation in the other tissues, the higher expression of PRLr observed in HSIL and CC suggest a possible role of signaling pathway of PRLr in the cervical cancer development. Partially supported by grant PROMEP 103.5/08/2919 and SEP-CONACYT 79709.

PC12/14 PRINCIPAL APPROACHES TO THE ELABORATION OF THE IMMUNOENZYME ANALYSIS OF AUTOANTIBODIES TO GAD AS A MARKER OF INSULIN-DEPENDENT DIABETES MELLITUS

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Autoantibodies to glutamic acid decarboxylase (Anti-GAD) are the informative marker for the revealing of individuals at high risk of insulin-dependent diabetes mellitus (IDDM) development. They are one of the first predictors of IDDM and can be detected 5-7 years before the clinical onset of the disease; their presence in the blood is the evidence of the autoimmune destruction of the pancreatic insulin-secreting beta-cells. The aim of the present work: development of the new express quantitative ELISA technique for the analysis of anti-GAD concentrations – marker of the autoimmune damage of the pancreas. In the basis of the elaborated method is ELISA format. Principle of the test: anti-GAD present in the patients' serum samples bind to GAD, immobilized onto microwells. An enzyme (alkaline phosphatase) labeled monoclonal antibodies, specific to human IgG, are added to the formed immunocomplex "antigen-antibody". A substrate of the alkaline phosphatase (PNPP) is used as indicator in this test. After stopping of the color reaction with NaOH it is measured spectrophotometrically, and the intensity of color reaction developed is directly proportional to the anti-GAD concentration in the sample.

Conclusion: Developed anti-GAD ELISA test allows to reveal with high precision and specificity the presence of even microquantities of anti-GAD and to diagnose the initial stages of autoimmune damage of pancreas and IDDM, and thus gives the investigators the precise quantitative criterion for the early IDDM diagnostics, control of the pathological process expression degree, estimation of the therapy efficacy and prognosis of the disease development.

PC12/15 IDENTIFICATION OF IMMUNE MARKERS ON THE SURFACE OF THYROID FOLLICULAR CELLS FROM PATIENTS WITH GRAVES' DISEASE USING CELLULAR CULTURE

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In the pathogenesis of Graves' disease an important role play proinflammatory cytokines. They influence on immune system and also on destination cells by induction of antiapoptotic molecules expression causing resistance of thyrocytes to apoptosis – the programmed cells death, coursed by CD 95. The aim of the study was to detect the expression of CD 54, CD 95, CD 134, CD 152 in the thyroid tissue in patients with Graves' disease (GD) and patients with non-toxic nodular goiter (NTNG) during the cellular culture with or without cellular stimulators. Investigated thyroid tissue was cleaned and then mechanically and enzymatic fragmentation using collagenase with HBSS. So obtained suspension of cells was pass through the nylon-filter. The culture of thyroid cells managed into 50 mls phials in the basis of cultivation containing: RPMI 1640, 10% FBS, HEPES buffer, L-the glutamine, the penicillin and the streptomycin. After isolation of thyrocytes (3x10⁵) the culture was managed into 6-pit plates through the period of 5 days. In to the culture was added TNF- α IL-1 β and INF- γ . Identification of CD 54, CD 95, CD 134, CD 152 markers on thyrocytes, before and after use of cellular stimulators was performed using flow cytometry on apparatus Coulter XP.

The analysis of CD 54, CD 95, CD 134, CD 152 markers expression on thyrocytes showed their elevation in group of patients with GD in comparison to group of patients with NTNG. During the cellular culture percentage of cells with CD 54 and CD 95 expression significantly decreased. Application of cellular stimulators led to the height of the expression CD 54, CD 134 and CD 152 in both examined groups. In conclusion, changes of the expression of CD 54, CD 95, CD 134, CD 152 molecules on the thyroid follicular cells suggest the different degree of the activation and stimulation of the cells during the development of the pathological process within thyroid gland.

PC12/16 SERA CONCENTRATIONS OF ANTI-THYROPEROXIDASE AUTOANTIBODIES MEASURED BY TWO RADIOIMMUNOASSAYS

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Measurement of autoantibodies against the thyroid microsomal antigen, now identified as thyroperoxidase autoantibodies (TPOAb), is necessary in diagnosis of autoimmune thyroid diseases, especially Hashimoto's thyroiditis. There are a lot of commercial assays for determination TPOAb in patients' sera. The aim of this study was to compare TPOAb concentrations obtained by using two radioimmunoassays (RIA): *CIS Bio international* (France) and *Immunotech* (Czech Republic). Blood samples (5 ml) for measurement of TPOAb concentrations were collected in the Clinical Centre Kragujevac. After allowing the sample to clot, the serum was separated by centrifugation at 3000 rpm for 15 minutes, decanted and preserved at -20°C until used. The total number of specimens was 38. Analyses were performed according to producers' manuals. The values of sera TPOAb concentrations obtained by two RIA assays were compared by regression analysis and the correlation coefficient $r = 0.61$ was obtained. There was significant difference between absolute values of TPOAb concentrations in almost all samples, and the higher absolute values were obtained by using *CIS Bio international* assay. Besides, the relative values (calculated as ratio of measured and upper limit concentrations) determined by *CIS* radioimmunoassay were higher than the values obtained by *Immunotech* assay. Despite the differences in absolute and relative values of TPOAb obtained by two RIA assays, high data in one test were proved by the other one, and we could say that the both assays we used are sensitive in the diagnosis of autoimmune thyroid disease.

Key words: TPOAb, radioimmunoassay, autoimmune thyroid disease

PC12/17 EXAMINATION RECIPROCAL RELATIONSHIP OF BLOOD PROFILE IN GREAT STURGEON (*HUSO HUSO*) CULTURED IN BRACKISH WATER

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Recognition of blood profile exchange and awareness of their reciprocal relationship is important in aquaculture, especially in recognition of disease, abnormal condition and determination time of injection. The present work reports on blood profile measured in 4-5 years old Great sturgeon cultured in brackish water pools in BAFGH-Iran. Blood sampling was performed in caudal vein every three month and plasma was frozen until future analyses. Hormone levels measured by Radio Immunoassay (RIA), Glucose with Authoanalyser, calcium and magnesium with spectrophotometer and sodium and potassium with film photometer, in Yazd central laboratory. Statistical result show that some biochemical parameter had direct significant correlation, include: glucose/ calcium; sodium/ potassium; sodium/ magnesium; potassium/ calcium and potassium/ magnesium, which with increase of someone, amount of other will be increase. Among hormonal profile, testosterone/ estradiol had direct significant correlation. Also Cortisol with glucose, sodium and potassium had direct significant correlation, but Cortisol hadn't significant correlation with other hormone and this correlation was invert. Eventually definite that blood relationship in Great sturgeon is like other sturgeons.

Keyword: Reciprocal relationship, blood profile, Great sturgeon, aquaculture, plasma.

PB14 – AGEING

PB14/1 A CRITICAL ROLE FOR EBF IN THE LOSS OF B CELL POTENTIAL OF HEMATOPOIETIC PROGENITORS WITH AGE

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Ageing is accompanied by a reduction in the generation of B lymphocytes leading to impaired immune responses. In this study we have investigated whether decreased B cell production is due to age-related defects in multipotent bone marrow hematopoietic progenitors. By in vitro limiting dilution assays we found a 2-5 fold reduction in the ability of hematopoietic stem cells from old mice to generate B cells, while myeloid potential remained unchanged. This age-related decrease in B cell potential was more marked (8-20 fold decrease) in common lymphoid progenitors (CLP) and was associated with reduced expression of the B-lineage specifying factors, EBF and Pax5. Notably, retrovirus-mediated expression of EBF complemented the age-related loss of B cell potential in CLP isolated from old mice. Furthermore, transduction of CLP from old mice with a constitutively active form of STAT5 restored both EBF and Pax5 expression and increased B cell potential. Taken together, these data show that reduced EBF expression, perhaps due to impaired STAT5-mediated IL-7 signaling, plays a critical role in the loss of B cell potential of hematopoietic progenitors with age, leading to a decline in B cell generation.