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SOLUBLE INTERLEUKIN-6 RECEPTOR IN RHEUMATOID ARTHRITIS (RA) AND SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Soluble interleukin-6 receptor (sIL-6R), wich occurs is biological fluid, can make soluble complex with its ligand, interleukin-6 (IL-6), and that binds to gp-130, making IL-6R negative cells sensitive for IL-6. That way sIL-6R plays agonistic role. Its generation and function is poorly understood in autoimmune diseases.
SIL-6R levels were measured by Elisa sandwich technology in sera and in supernatants of lymphocyte cultures, treated or untreated with dexamethasone. IL-6R mRNA was measured by RT-PCR.
SIL-6R levels in sera of patients with active RA and inactive SLE were higher than those of healthy control, inactive RA and active SLE group. In vitro dexamethasone treatment stimulated generation of sIL-6R in healthy and SLE persons, and strongly supressed it in both RA group. Both the mRNA coding the membrane anchored and soluble IL-6R transport increased. The strong decrease found at sIL-6R protein hasn't been detected at mRNA level.
SIL-6R is detectable in sera and lymphocyte culture supernatants of RA and SLE patients and healthy controls. Its level depends on disease itself and its activity.

PROLACTIN AND INTERLEUKINS (ILs) IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) DURING THE TREATMENT WITH QUINAGOLIDE (NORPROLAC)

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The relationship between neuroendocrine regulation and the immune system has recently become the subject of intense investigations. Because hyperprolactinaemia has been implicated in the pathogenesis of SLE we investigated under standarized conditions the levels of prolactin, IL-1ra, IL-6, IL-10 in serum of 22 patients with SLE with various levels of disease activity and in 18 healthy controls with similar age and sex distribution and additionally we estimated the effects of quinagolide (from 25 μg to 50 μg pro die) on the levels in serum of this hormon and ILs studing their levels before and after 3, 6, 9 months of treatment. Basal prolactin levels were determined by quantitative immunoradiometric assay and concentrations of ILs were determined using human IL-1ra, IL-6 and IL10 immunoassay for the quantitative determination of concentration in serum. At entry, serum prolactin levels in the total group of patients with SLE did not differ statistically from control group (in patients - 403.00 ± 292.68 mIU/l, in control group-299.39 ± 137.11 mIU/l). Prolactin levels in quinagolide treated subjects were significantly lower after 3, 6 and 9 months of treatment than the levels before therapy. IL-6 concentration was elevated at entry in observed group of patients in comparison with control group (respectively 14.16 ± 13.02 pg/ml and 5.04 ± 3.35 pg/ml). After 3 months and especially after 6 months of treatment the concentration of IL-6 was statistically significantly decreased when compared before and after therapy with quinagolide (respectively 15.79 ± 15.11 pg/ml before and 4.30 ± 2.51 pg/ml after 6 months therapy). Our results suggest a causal relationship between the level of IL-6 in the serum and the quinagolide treatment used by several months in patients with SLE.

Lupus-like syndrome in the course of prolactinemia

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The case of lupus-like syndrome in women with long-lasting galactorrhea is presented. The case has been observed for 22 years. The symptoms of autoimmunological disease were observed 4 years after last delivery they had been followed by galactorrhea and is ceased spontaneously after menopausa. We were not able to define the causing factor of hyperprolactinemia in patient.

Key words: prolactin, galactorrhea, systemic lupus erythematosus, autoimmunization.

PREGNANCY OUTCOME AND ANTI SSA-Ro 52 kDa ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Objective: Increased incidence of adverse pregnancy outcome was repeatedly reported in patients with systemic lupus erythematosus (SLE) with anti-SSA-Ro Abs, and a hypothesis of intrauterine expression of neonatal lupus syndrome was presented. The aim of this retrospective study is to explore the problem in relationship to anti SSA-Ro 52 kDa Abs in SLE patients from the population of the East Bohemian region.

Methods: The group under study consists of 60 SLE women patients according to ACR classification (1982, updated 1997). For the determination of anti SSA-Ro 52 kDa Abs an immunobloting analysis was used by commercially available ANA/AMA IMMUNOBLOT diagnostic kit (DPC Bierman GmbH, Germany). Data with relationship to pregnancy outcome were obtained using standardised questionnaire concerning information about pregnancy and reproduction. In addition, a screeening of SLE severity was made according to frequency of IV pulse therapy (methylprednisolone or cyclophosphamide), and cyclophosphamide p.o. The data were statistically processed using Fisher's exact test.

Results: 27 SLE patients were anti SSA-Ro kDa Abs positive, and 33 negative. No significant differences ($p>0.05$) were found between both subgroups in the following parameters: No. of deliveries, neonates and their birth weights (all neonates were healthy), spontaneous abortion, hormonal contraception, age at the time of menarche, menopause, SLE clinical manifestation, and start of therapy. IV 1 g methylprednisolone pulse therapy and cyclophosphamide p.o. were more frequent in SLE with anti SSA-Ro 52 kDa Abs ($p<0.01$, respectively $p<0.05$).

Conclusions: In pregnancy outcome and other parameters associated with reproduction no significant differences were found between anti SSA-Ro 52 kDa positive and negative SLE women. The history of more frequent therapy using IV methylprednisolone pulses and cyclophosphamide p.o. suggests a more severe form of SLE in anti SSA-Ro 52 kDa positive subgroup of patients.

Parameters of cellular immunity in patients with rheumatic diseases.

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Immune response shows no pronounced alterations in pregnancy. Both antibody and cellular immunities are maintained. Since the immune mechanisms plays a role in the pathogenesis of rheumatic diseases, we monitored the changes in selected parameters of cellular immunity in 5 pregnant patients with rheumatic diseases (2 SLE patients, 2 PsA patients and 1 JCA patient) in the 6th and 9th months of pregnancy, a day after delivery and 2 months after delivery. The evaluated parameters were: subpopulations of leucocytes in peripheral blood, their activity and both phagocytic and microbicidal activity of polymorphonuclear leucocytes (PMNL).

Statistically significant increase of the PMNL phagocytic activity was found in patients 2 months after delivery. In the course of pregnancy, slight increase of T-lymphocytes, especially of helper CD4 lymphocytes was observed, as well as decrease of cytotoxic CD8 lymphocytes. After childbirth the values of all these parameters went back to the standard. There were no alterations observed in the numbers of NK cells either in the course of pregnancy or after delivery. The expression of the CD69 and CD25 receptors on the lymphocytes stimulated with phytohemaglutinin and CD2/CD2R monoclonal antibody decreased in pregnancy.

A possibility that, observed alterations in the cellular and non-specific immunities can contribute in the immune homeostasis disorder in rheumatic patients after delivery cannot be excluded.

ANTICARDIOLIPIN ANTIBODIES IN SLE

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Objective: In the study **the relationship between the occurrence of anticardiolipin (ACLA) and intracellular antibodies** in connection with thrombosis in SLE patients was studied.

Patients and Methods: 64 SLE patients fulfilling the ARA/ACR criteria were included in the trial. ACLA and anti-DNP antibodies were determined by ELISA methods. Anti-dsDNA antibodies were detected by Farr's method. Anti-ENA antibodies were studied by counterimmunoelectrophoresis, Western blot and ELISA.

Results: A statistically significant **increased frequency** of the occurrence **of anti-U1+Sm antibodies (RR=4.5)** was found **in the group of patients with positive ACLA** levels (> 20U/ml, tab.). Associations with the other anti-ENA antibodies, anti-dsDNA and anti-DNP have not been proved. **The correlation with the clinical parameters assessed confirmed the antiphospholipid syndrome (APS) in 5 patients (45.5%).** Only low and clinically asymptomatic ACLA titers were found in 3 other patients (27 %).

Conclusions: *The present study suggests that SLE with the presence of ACLA is markedly associated with anti-U1+Sm antibodies. The study confirms that there is a high probability of the development of APS in patients with high persisting ACLA titers.*

Antibodies Anti-	Frequency of antibodies in patients ACLA+ (n=11)		ACLA- (n=53)		chi²	P
U1	18.2 %	(2)	20.8 %	(11)	0.04	n.s.
Sm	0 %	(0)	3.8 %	(2)	0.43	n.s.
U1+Sm	**36.4 %**	(4)	**11.3 %**	(6)	4.33	**< 0.05**
Ro/La	18.2 %	(2)	28.3 %	(15)	0.47	n.s.
Ro	0 %	(0)	11.3 %	(6)	1.37	n.s.
rRNP	0 %	(0)	3.8 %	(2)	0.43	n.s.
Undefined	27.3 %	(3)	20.8 %	(11)	0.10	n.s
dsDNA	36.4 %	(4)	30.2 %	(16)	0.16	n.s.
DNP	72.7 %	(8)	71.7 %	(38)	0.01	n.s

ANTI-T AND ANTI-B LYMPHOCYTOTOXIC AUTOANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Objective: In our previous studies we found that **lymphocytotoxic autoantibodies (auto-LCA) are present in 64%** and lymphocytotoxic alo-antibodies in 90% **of SLE patients.** Auto-LCA were more specific. Their presence correlated with some antibodies in SLE. In the literature there are practically no data on the frequency anti-T and anti-B auto-LCA in SLE .

Patients: **43 SLE patients** (38 females, 5 males, ARA diagnostic criteria)

Methods: • isolation of T and B lymphocytes – immunobeads (Dynabeads)
• microcytotoxicity test – fluorescent modification at 37°C (warm reactive, IgG) and at 15°C (cold reactive, IgM); *auto-LCA– patient's cells and serum*
• percentage of T and B lymphocytes – double colour flow cytometry

Results:
• **IgG auto-LCA** were found in **4.8%** of SLE patients (2/42). All antibodies were against B lymphocytes. This patients suffered from clinically active forms of SLE with marked leukopenia (<2500), T-lymphopenia (<600) and B-lymphopenia (<90).
• **Anti-T IgM auto-LCA** were found in **46.5%** (20/43) and **anti-B IgM auto-LCA** in **63.4%** (26/41) of **SLE patients.**
• Significant relationships were found between the occurrence of anti-T auto-LCA (IgM) and T-lymphopenia (<1100) and anti-B auto-LCA (IgM) and B-lymphopenia (<200). **51% of SLE patients had T-lympho-penia** and 73% of them had anti-T auto-LCA (IgM). **77% of SLE patients had B lymphopenia** and 83% of them had anti-B auto-LCA (IgM)
• **The presence of anti-T and anti-B auto-LCA (IgM) correlated with some antibodies in SLE** (table). No relatioships were observed in butterfly erythema, lupus nephritis, arthritis and the lung, CNS and heart involvement.

Conclusion: *The examination of lymphocytotoxic anti-T and anti-B autoantibodies in SLE can be helpful in the clinical practice.*

Laboratory Finding	Frequency of laboratory finding in anti-T auto-LCA (IgM) posit.	negat.	P	anti-B auto-LCA (IgM) posit.	negat.	P
anti-DNP	84%	32%	<0.001	70%	36%	<0.05
anti-dsDNA	47%	16%	<0.05	42%	15%	n.s.
anti-ENA	100%	53%	<0.001	86%	60%	n.s.
CH50 (<25)	55%	18%	<0.01	52%	7%	<0.05

Lupus nephritis patients outcome after discontinuation of 2-year treatment with cyclosporine versus cyclophosphamide (comparative pilot study)

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Objective. To determine the outcome of patients (pts) with active lupus nephritis (LN) after discontinuation of 2-year therapy with cyclosporine A (CsA) or cyclophosphamide (CPM). **Methods.** LN verified by repeated renal biopsies was treated with CsA in 11 pts (Group I) and with CPM in 13 pts (Group II) for 2-year period. Initial renal biopsies revealed focal proliferative LN (class III acc. WHO) in 6 pts, diffuse proliferative (class IV) in 14 pts and membranous (class V) in 4 pts. Control renal biopsies after 1 year therapy performed in 14 pts evidenced distinct improvement with decrease of the activity signs in all pts investigated (Arthritis Rheum Abs. Suppl.1997:40: S57; Lupus 1998: 7: 29-36). CsA was aministered in oral form at maintenance dose of 2.5 to 3.5 mg/kg/day and CPM was given in continuous oral or i.v. pulse form in standard doses. **Results.** The summarized outcome of all 24 enrolled pts after discontinuation of the therapy is demonstrated :

No of patients	Renal remission A	B	C	Renal exacerbation D	Withdrawn from the study
CsA (Group I) (n = 11)	9 / 11	4 / 9	4 / 9	5 / 9	** 2 / 11
CPM(Group II) (n = 13)	10 / 13	6 / 10	* 2 / 10	4 / 10	*** 3 / 13

A: At the monent of discontinuation of 2-year therapy (CsA / CPM)
B: Remaining for consecutive 1 year after discontinuation of 2-year therapy
C: Remaining for consecutive 2 years after discontinuation of 2-year therapy
*4 pts will reach 2 years after discontinuation of primary therapy in 2000
D: Necessity of restoration of primary therapy (CsA / CPM in consecutive 2 months. **no cooperation / lung Ca, ***toxic leucopenia / hemorhagic cystitis / recurr. pneumonia

Conclusions. No substantial and statistically significant differences in the efficacy of 2-year long therapy of LN with CsA or CPM as well as in consecutive 2-year period characteristics after discontinuation of treatment were established in 24 monitored patients.

TEN YEARS EXPIRIENCE WITH HIGH "PULSE" DOSES OF CYCLOPHOSPHAMIDE IN THE TREATMENT OF THE MOST SEVERE SYSTEMIC LUPUS ERYTHEMATOSUS

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In last ten years (1988-1999) in our Clinic we treated 178 patients (162 women and 16 men) with diagnosis of systemic lupus erythematosus according to revised ARA criteria. Mean age of onset of disease was 26 years. The most severe forms of disease were noticed in 54% (96/178) patients. Lupus glomerulonephritis was diagnosed in 78% patients (75/96), severe skin vasculitis in 19% (18/96), while 13% (12/96) among them developed carditis.

In the treatment of 71% (68/96) patients with these severe forms of diseases we performed high "pulse" doses of cyclophosphamide (Cy). At the beginning of cyclophosphamide therapy they were aged 14-65, mean 31.3 years. Therapeutic protocol contained of intravenous infusion of 0.5-1 g Cy in psysiologic solution together with 40 mg methylprednisolone which was always preceded and followed by huge hydration (2,5-3 l). This protocol was applied monthly during first 6 months and then 6 times in 3-month-interval. In 75 % patients (51/68) we obtained complete remission of disease activity and in 10% (7/68) incomplete remission. Therapy failed to reduce disease activity in 15% patients (10/68). Relapse of primary disease was registered in 13% (9/68) patients in period of 1-7 years (mean 2.5). As adverse effects we recorded bacterial and viral infections in 25% (17/68) and development of terminal kidney insufficiency in 6% (4/68) patients. During the therapy 10% (7/68) patients died: due to primary disease - SLE (4 patients), lung carcinoma (1 patient), sepsis (1 patient) and cerebrovascular insult (1 patient).

Our results clearly showed that high "pulse" doses of cyclophosphamide in the treatment of the most severe systemic lupus erythematosus are efficient and (probably due to crucial rehydration) well tolerated.

CRYOGLOBULINAEMIC VASCULITIS

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Cryoglobulins are immunoglobulins who precipitated in the low temperature. Cryoglobulinaemic vasculitis is inflamatory disease of small vessels elsewhere of the body, mostly in kidneys, joints, skin and peripheral nerves, causing glomerulonephritis, arthritis, purpura and peripheral neuropathy.

Aims of this study was:

1. To show incidence of cryoglobulinaemic vasculitis between other vasculitic syndromes,
2. To find out the spectrum of clinical manifestations,
3. To show the importance of connection with hepatitis B virus infection.

We investigated 39 patients /30 women 19-72 X=47,26 yr., and 9 man from 44-64, X=60,10 yr./. All fulfilled diagnostic criteria for this form of vasculitis.

Cryoglobulins are measured by turbidimetric method, components of complement with radial immunodifusion, and VHB and their antibody with ELISA method.

We find out skin changes /purpura/ all of patients, high temperature in 26 /66,66%/, arthritis in 25 /64,10%/, glomerulonephritis in 11 /28,10%/ and neuropathy in 21 /53,84%/, Sy Raynaud was found in 15 /38,46%/.

All of patients have had high values of cryoglobulins /100%/ hypocomplementemia C-3 in 13/39 /33,33%/, C-4 22/39 /56,41%/, RF 18/39 /46,15%/. Hepatitis B virus /HBV/ was found in cryoprecipitate only in 13/39 /30,95%/ patients.

We concluded that cryoglobulinemic vasculitis is confirmed in 39 out of 308 patients /12,55%/. The most commonly manifestations of illness are purpura, arthritis, polyneuropathy and glomerulonephritis. Behind VHB who is find out in 30,95% patients it seems /from the literature data/ that VHC is the most important cause of cryoglobulinemic vasculitis.

METHOD OF INDUCED SPUTUM IN SCREENING OF PULMONARY INVOLVEMENT IN CONNECTIVE TISSUE DISEASES

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Pulmonary involvement is frequent and serious organ manifestation of connective tissue diseases. There are some diagnostic possibilities – repeated estimation of diffusion lung capacity, high resolution CT scan,bronchoscopy with exploration of bronchalveolar lavage . Some of these methods represent stress for patient, others are expensive for repetitive use.

In diagnosis and management of bronchial asthma plays important role method of induced sputum. It´s simple, non-invasive,reproducible method, measuring intensity of inflammatory and allergic reaction.

There are anecdotal references ,using this diagnostic method in intersticial pulmonary involvement.

We used method of induced sputum in group of patients with connective tissue diseases, suspected from active pulmonary involvement.

Patients and methods: 32 patients with established diagnosis of connective tissue diseases , fulfilling classification criteria of ACR in rheumatoid arthitis, systemic lupus erythematosus, or systemic scleroderma ,eventually Bohan´s and Peter´s criteria for dermato/polymyositis, were examined for pulmonary disease . Induced sputum were obtained after inhalation of 5 ml 3percent solution of natrium chloride by ultrasonographic nebuliser. The induced sputum were cultivated, and made cytological and immunofenotypisation examinations.

We discusse first results in technical succes, cytological and immunotypisation results, comparing with results of traditional methods.

Conclusions: Method of induced sputum seems , after thoroughgoing appreciation,the interesting possibility of easy,non-invasive and repetitive method for screening of pulmonary involvement in connective tissue diseases.

PYRIDINOLINE CROSS-LINK IN FIBROTIC TISSUES IN PATIENTS WITH SSc

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Systemic sclerosis (SSc) is a connective tissue disease of unknown actiology associated with the excessive accumulation of collagen in the skin and internal organs. Mature collagen cross-link pyridinoline (Pyd) occurs in significant amounts in cartilage, ligament, tendon and bone collagens. Increased levels of Pyd were recently found in the urine of SSc patients. However, the tissue origin of excessive urinary Pyd still remains unclear.

The aim of our study was to determine the content of Pyd in fibrotic tissues of different origins (skin, fascia, endocardium and bladder autopsies) in patient with systemic sclerosis and to compare it with normal tissues of the same origin.

Pyd and hydroxyproline were determined by reverse-phase HPLC methods. The concentrations of Pyd in the tissues were expressed as the relative amount of Pyd per mol of collagen, assuming that each molecule of collagen contains approximately 300 hydroxyproline residues.

The significant increase of Pyd was found in the fascia, endocardium and bladder. The concentrations of Pyd in the fibrotic skin samples were more than twice as high than in samples of normal skin. Skin, the biggest organ which undergoes extensive fibrosis in SSc, contains large amount of collagen. Thus, despite the small density of Pyd in fibrotic skin, it could dominantly affect the urinary concentration of Pyd.

Tissue	Pyd concentration (mmol/mol collagen)	
	Normal	Fibrotic
Skin	30	70
Endocardium	690	980
Fascia	990	1250
Bladder	290	770

The results of our study clearly show the increased amount of Pyd cross-link in collagen fibrils in SSc patient. Tissue fibrotisation in SSc patients seems to be one of the conditions leading to the accumulation of pyridinoline cross-link, even in tissues as normal skin with low concentrations of this type of cross-link.

HYPOBARIC THERAPY OF PATIENTS WITH SCLERODERMIA

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Sclerodermia is chronic systemic disease of connective tissue, which affects the skin and inner organs of the body. Patients suffer from several symptoms that can be treated also with physical therapy:
- microcirculation disturbances on extremities
- galvanisation, CO2 baths, lymphatic drenage and massage
- symptoms of sensory neuropathies
  - several balneotherapeutic procedures
- connective tissues hardness
  - individual kynesitherapy, hypobaric therapy
- osteoarticular and myofascial pain
  - analgesic modalities of physical therapy

The presented research is showing therapeutic possibilities of hypobaric therapy in treatment of patients with sclerodermia.
We treated 8 patients with akrosclerodermia who suffered from fibromyalgic pain. They were all women, age from 42 to 48 years. Hypobaric therapy was applied by mean of computer guided vacuum massage - the value of underpressure and time of application were determined individually. The clinical parameters and laboratory test (CPK, CRP, serum lactate) were followed. We used digital lactatometre to determine values of serum lactate before and after the hypobaric treatment of each patient.
The results showed statistically significant ($p<0.05$) better range of movement in affected joints, longer distance of claudication and lower concentrations of serum lactate in patients after the treatment with hypobaric therapy.
The results confirmed positive action of hypobaric therapy on improved oxygenation of affected tissues in human body.

LABORATORY FINDINGS IN THE "THROMBEMBOLIC" AND "NON-THROMBEMBOLIC" SUBGROUPS OF PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES

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Antiphospholipid antibodies (APA) represent a group of extraordinary heterogeneous antibodies with various influence on blood coagulation system. APA could be associated with arterial and venous thrombosis, recurrent pregnancy loss, and thrombocytopenia in antiphospholipid syndrome. It would be of high importance for treatment and/or prevention in risk situation, if "thrombogenic" and "non-thrombogenic" APA could be determined by tests available for the clinical practice. The aim of our study was to establish if examination of coagulation and fibrinolytic system could be useful in this cases and which of them.

Since May 1997 to October 1999 341 healthy persons from blood donors, 125 patients with rheumatic disease and 85 patients with malignancy were evaluated for APA presence. We evaluated prothrombin split product (F1+2), plasminogen-antiplasmin (PAP) and thrombin-antithrombin (TAT) complexes and D-Dimer (assays Behring:Enzygnost F1+2, Enzygnost TAT, Enzygnost PAP, Enzygnost D-Dimer) in 82 patients (52 women, 30 men) with positive antiphospholipid antibodies (assays Stago:Asserachrom APA, APA IgG IgM, PTT-LA, dRVVT, KCL, Staclot LA) and in a group (20 women, 15 men) of healthy persons without APA . APA positive persons were divided into subgroup with positive personal history of thrombosis, pulmonary embolism and/or repeated foetal loss and subgroup without any thrombembolic event. Comparison of the "thrombembolic" and "non-thrombembolic" APA positive subgroup did not prove difference in evaluated markers. Any significant differences between APA positive and control group in F1+2 and TAT have not been found. We found significant differences in D-Dimer and PAP, which could have been partially explained by „older" patients group and by underlying disease in presence APA.
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REFRACTERY THROMBOCYTOPENIA IN A PATIENT WITH SECONDARY ANTIPHOSPHOLIPIDE ANTIBODY SYNDROME

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Thrombocytopenia is an important clinical sign of antiphospholipide antibody syndrome (APS). Severe thrombocytopenia ($<10 \cdot 10^9/l$) appears in less than 5% of patients with systemic lupus erythematosus (SLE). Some patients with SLE, APS and severe thrombocytopenia are resistent to standard therapy.

This is a case report of a woman, who was born in 1952, has had epilepsy since her childhood and primary arterial hypertension since she has been 26 years old. When she was 41 years old, she had cerebrovascular accident. She has never been pregnant although she wanted to and hasn't had vascular thrombosis. When she was 45 years old, there was fatigue, non-erosive arthritis, ANA/IF positivity, anti ds-DNA antibodies in abnormal titre, higher serum level of anticardiolipin IgG antibodies (aCL IgG Abs); MRI of the brain showed chronic cerebral cortex and subcortex infarct in right temporooccipital region, MRI angiography showed old signs of arteritis of insular branches of right arteria cerebri media. This woman met 4/11 (1982, 1997) ACR classification criteria for SLE in January 1998 and the therapy with hydroxychloroquin was started; because of higher titres of aCL IgG Abs we went on the therapy with acetylosalicylic acid.

In June 1998 the patient came with leucopenia, anemia, thrombocytopenia ($5. 10^9/l$) and haemorhagic syndrome. aCL IgM + IgG Abs and anti β2 glycoprotein 2 antibodies were present in high titres. There was flare of SLE and signs of secondary APS. In this situation i.v. therapy with four 1 g pulses of methylprednisolone was administered; then we continually went on with glucocorticoids p.o.; we gave also IVIG (870mg/kg/6 days), then she had plasmapheresis 7 times. As serious thrombocytopenia ($10 . 10^9/l$) was still present, we started the therapy with cyclosporin A (CsA) – 3.8 mg/kg/day. Although there were some relative contraindications for therapy with CsA (compensated arterial hypertension, using of carbamazepin), the level of thrombocytes went continually up, in January 2000 she had $140 . 10^9/l$ (1.8 mg CsA/kg/day) and no adverse effects of the therapy appeared so far.

Data in this case report suggest, that cyclosporin A in a patient with SLE, APS and refractery thrombocytopenia could significantly increase serum level of thrombocytes for quite a long time without side effects of such treatment.

SALMONELLOSIS AND SLE

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Objective: Nowadays, when SLE patients' survival is longer and, due to immunosuppressive therapy the concurrent lupus nephritis and CNS involvement can be succesfully supressed, infectious complications occur more frequently since secondary immunodeficiency often develops during the long immunosuppressive therapy. Infectious complications develop not only in the active stage of SLE, but also in the long-term course of the disease. Salmonella infections are responsible for 40 % of all bacteremia in SLE. The prognosis in nontyphoid extraintestinal salmonella infection in patients with SLE is generally regarded as bad with mortality of 20-28.5 %.

Methods: We present 5 cases of successfully treated salmonella complication in female patients with SLE, aged from 19 to 46 years (median 25 years), with disease duration from 12 months to 7 years (median 16 months). All patients fulfilled revised ARA criteria for the SLE. All of them were administered long-term immunosuppressive treatment (glucocorticoids, cyclosporine A, cyclophosphamide) for the active SLE form, in 4 cases with lupus nephritis and in one case also with serious CNS affection and in one case with pleuritis

Results: *Salmonella enteritidis* was detected as causative agent in all the patients. In 4 cases it was verified via blood/urine cultivation and in one indirectly via serological evidence of antibodies. In the clinical picture were febrilities dominated, in 4 cases of septic character with trembles. We found out gastroenteritis 3-times, urinary infection 1-time, pneumonia 1-time, 3x bacteremia 4-times and abscess 1-times.

Conclusion: Authors emphasize the importance of early introduced immunomodulatory therapy enabling them in most cases, besides the effectively and sufficiently administered antibiotics, also the prevention of septic complications in SLE.

SALMONELLA INFECTION WITH NEGATIVE ANTI-BODIES IN AN SLE PATIENT.

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Secondary infections are among the most frequent complications in SLE patients, often with fatal consequences. The second most often detected microbial agent after *Staphylococcus aureus* are Salmonella, mostly non-typhoid, infections.

In our paper, the course of disease, dominated by skin and joint symptoms, is described in a 25 years old SLE female patient. In 1995 patient developed SLE, the course of disease was complicated by gastro-enteritis in 1998, during which *Salmonella enteritis* was cultivated. In the summer 1999, the patient was hospitalised in our Institute (RIRD) with both clinically and immunologically proven flare-up of SLE (interstitial pneumopathy, lupus exanthema, episodic arthritis, fevers, ANA 2+H, anti-DNP 215, anti-dsDNA 14, CH50 0). Serological proof of the antibodies to Salmonella of the IgG and IgA classes (ELISA) was negative. No antibodies of either of the classes were detected even during septicaemia that appeared later during hospitalisation, when *Salmonella enteritis* was cultivated from the blood and rectum samples. Intensive antibiotic therapy followed by glucocorticoid pulses suppressed the infection and gradually clinical signs of SLE as well. At the time of a check-up examination in November 1999, both the cultivation test and serological test of antibodies to Salmonella were negative.

This case report underlines the relevance and importance of direct proof by cultivation of the infection agent in spite of repeatedly negative serologic examination. The false negative serological testing to Salmonella could have easily led a physician to misdiagnosing with possible fatal outcome for the patient.

TRACHEOBRONCHIAL RELAPSING POLYCHONDRITIS A CASE REPORT FROM 7-YEAR FOLLOW UP.

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Relapsing polychondritis (RPC) is a rare disease characterised by inflammation of cartilaginous structures with respiratory involvement in 30 % - 50% of referred cases. The occurrence of antibodies to collagen II suggest an autoimmune mechanism of pathogenesis. Activity of disease is usually accompanied with elevated sedimentation rate (ESR). Treatment includes NSAID, corticosteroids and cytotoxic agents.
We report a case of tracheobronchial RPC with the slow onset but severe irreversible airflow obstruction and tracheal bifurcation stricture introduced by pulmonary infiltrate suspected from mycobacterium aetiology in 40 year old woman. The symptoms of fatigue, weight loss, cough and dyspnea didn't resolve after successful antituberculotic treatment. The diagnosis of RPC was approved by typical histology picture of ear biopsy and by bronchoscopy and HRCT findings of mayor airways collapse. The corticosteroid and cyclophospamide (CFA) therapy led to remission in 2 months. The attempt of withdrawing CFA was followed by relapse manifested with laryngeal subglotic stenosis and progression of respiratory obstruction (see table). This course has required resumption of low dose CFA and corticosteroid therapy.

Spirometry findings between 1993 and 1999

Disease activity	VC *L (% predicted)*	FEV1 *L (% predicted)*	MMEF *L/sec (% predicted)*
Onset	3,29 (93,7 %)	1,12 (36,9 %)	0,7 (19,2 %)
Relapse	2,68 (76,8 %)	0,48 (15,9 %)	0,25 (6,8 %)
Remission	3,11 (90,4 %)	0,6 (20,8 %)	0,3 (8,63 %)

We did find the rising titres of anti collagen antibodies in the time of relapse but surprisingly anti type I and III (using ELISA method).
Anti II type collagen antibodies were bordering or even negative.

Titre of anti collagen antibodies and ESR

Activity	type I	type II	type III	ESR
Onset	1 : 256	1 : 32	1 : 64	72 / 105
Remission	1 : 64	1 : 16	1 : 16	7 / 22
Relapse	1 : 2048	1 : 8	1 : 128	64 / 120
Remission	1 : 64	1 : 64	1 : 16	15 / 35

The long term immunosupression and the pattern of anti collagen antibody response in the referred case are discussed.

THERAPEUTIC PROBLEMS IN SYSTEMIC LUPUS ERYTHEMATOSUS AND SELECTIVE IGA DEFICIENCY (CASE REPORT)

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The Selective IgA Deficiency in systemic lupus erythematosus (SLE) patients is 10-15 times more frequent than in general population. When a patient with SLE and Selective IgA Deficiency needs aggressive immunosuppresion or has a serious infection, there could be a problem in management, which is demonstrated in this case report.
43 years old woman with SLE was observed for 32 month. She meets 9/11 Classification criteria for SLE/ACR (1982, 1997) - higher photosensitivity, mouth ulcerations, non-erosive arthritis, proteinuria, grand mal seisures, haemolytic anemia, lymphocytopenia, anti-ds-DNA and ANA/IF in abnormal titres.Nephrobiopsy showed mesangial proliferative glomerulonephritis. This women has also interstitial lung fibrosis with reduction of diffuse lung capacity to 35 % of normal values. In serum she has normal IgM and IgG, but low IgA level (0.07-0.10 g/l).
Management of this patient with SLE and multiorgan involvement started with three 1 g i.v. pulses with 6-alfa-methyl-prednisolon, then oral glucocorticois and i.v. pulses with cyclophosphamide (15 mg/kg/month). The treatment was complicated by subletal bronchopneumonia which was treated with antibiotics. We didn't give polyvalent immunoglobulins in this situation because of the risk of possible anaphylactic reaction in a patient with Selective IgA Deficiency. After bronchopneumonia we change i.v. cyclophosphamide to oral azathioprine (1-2mg/kg/day) for a year. After that nephrotic syndroma with proteinuria more than 6 g/24 h. appeared That's why we change oral azathioprine again to i.v. cycylophosphamide, but in lower dose (8 mg/kg/month). We are going on with prednisolon oraly 0.5 mg/kg/day. On this last therapy is the patient without flare and without severe infecion for last 8 month.
The managenet of a patients with SLE and Selective IgA Deficiency could be difficult, when there is a need for aggressive immunosuppression or during severe infections, as the immunosuppression of such patients is deeper and the use of polyvalent immunoglobulins is contraindicated.

Vaccination of the imunocompromised patient

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The increasing number of immunocompromised persons are at higher risk of acquiring certain infectious diseases. Health profesionals need to implement adequate vaccination strategies. According to the type of the underlying disease and itś therapy the response may be complicated or suboptimal. The work contents clear recomendation to the physicians about strategy of immunization with different vaccines , itś risks and benefits. It helps to find alternative solutions in the case of ineffectivity of standard procedures to provide longlasting protection.

TREATMENT OF IMMUNODEFICIENCIES, SEVERE BACTERIAL INFECTIONS AND SOME AUTOIMMUNE DISEASES WITH HUMAN INTRAVENOUS IMMUNOGLOBULINS

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Since 1988 we have been used human intravenous immunoglobulin preparations (hIVIG) in the therapy of immunodeficiencies, severe bacterial infections and some autoimmune diseases. The aim of study was evaluation of hIVIG effects on these patients.

In mentioned period we have treated 8 patients with primary as well as 5 patients with secondary immunodeficiencies (excluding AIDS). 7S hIVIG (200-400 mg/kg/day every 3-4 weeks) were applied in all patients. Frequency and hardness of infections were significantly reduced in 92% (12/13) patients. Thus, except one patient who developed anaphylactic reaction, all patients considerably improved quality of life.

Patients with severe bacterial infections (No=23) were cured with 5S hIVIG (200 mg/kg/day during 3-5 days). Complete and fast recovery without any side effects was reached in 91 % (22/23) patients. One patient died of sepsis.

In 25 patients with autoimmune diseases, which were resistant to conventional immunosepressive therapy, we performed high "pulse" doses of 7S hIVIG (400 mg/kg/day for 5 consecutive days, every 4 weeks, during 2-6 months). Complete remission of systemic lupus erythematosus (total number 17 patients) was achieved in 76% (13/17) patients while 24% (4/17) patients died upon disease activity. Patients with primary Sjögren syndrome (No = 2), systemic vasculitis (No = 2) and idiopathic thrombocytopenic purpura (No = 1) were successfully treated with hIVIG with complete lack of disease activity. One woman with primary antiphospholipide syndrome (total number of treated patients was 3) has given birth to healthy child. In all these patients serious adverse effects of treatment were not noticed.

We concluded that treatment with hIVIG as substitution of immunoglobulins in immunodeficiencies, as adjuvant therapy in severe infections and as immunomodulation in some autoimmune diseases is very effective and well tolerated. Because of expensiveness, this mode of treatment should be reserved only for patients who failed to respond to conventional therapies, or patients suffering from life threatening diseases.

THERAPEUTIC MENAGEMENT IN WEGENER'S GRANULOMATOSIS (W.g.) PATIENTS

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Wegener's granulomatosis is one of the forms of necrotic systemic vasculitis that affects upper and lower respiratory tract as well as renal glomerules. Ethiopatogenesis of the disease remains unclear. Involvement of respiratory system suggests hypersensitivity to extra or intragenous antigen in the respiratory epithelium. Thus antibacterial drugs in addition to immunosuppresive treatment seems to be of great value.

Aim : To observe the efficacy of combined treatment (prednison, cyclophosphamide, trimetoprim/sulfametoxasol) in Wegener's granulomatosis patients.

Patients and methods : 6 patients with W.g. were observed (F : M = 4 : 2, aged from 31 to 52, mean 39.83). Disease duration lasted 2 to 144 months, mean 37 months. From the moment of the start of combined therapy the observation lasted 12 to 96 months. In all patients the diagnosis was made according to the ARA criteria and confirmed by typical changes in histopatological examination. As diagnosed all patients received treatment with prednison (not less then 1 mg per kg of body weight at the begining of the treatment), cyclophosphamide (1 g monthly i.v.) and trimetoprim/sulfametoxasol (960 mg twice daily oraly for 10 consecutive days each month).

Results : 5 of 6 patients presented inflammatory changes in upper respiratory tract as a first symptoms of the disease. Only one patient started medical history with pseudotumor of orbit. At the clinical examination all patients presented fever and respiratory tract involvement. Arthritis, nephritis and cardiac system involvement were observed in 4 patients, pharyngitis, sinusitis, medial otitis - in 3 patients. Two patients had central nervous system and one cranial nerves involvement. In 2 cases skin vasculitis was present.

All patients had elevated laboratory parameters of inflammation (ESR, seromucoid level). 4 of 6 patients had eleveted count of leukocytes and trombocytes. cANCA were present in 2 of 5 cases studied. None of patients had ANA nor anti - dsDNA antibodies.

The long - term efficacy of combined treatment will be discussed in details.

Conclusion : The combined therapy with prednison, cyclophosphamide and trimetoprim/sulfametoxasol is effective, safe and well tolerated.

ASSOCIATIONS OF PROFESSIONAL XENOBIOTIC AND IMMUNE DEPENDENT PRIMARY SYSTEMIC VASCULITIS

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The aim of current study was to determine Primary Systemic Vasculitis (PSV) development association with patients' occupation. Xenobiotic factors effect was assessed.

There were 350 patients with PSV, 210 males, 95 females, mean age 32,5±4,3 years, in disease during 5,8±1,9 years. 260 patients (74,28%) had occupational risk factors, 136 of them (52,3%) used to work with tetraethyllead (TEL). Immuno-enzyme, immuno-chemical and cytometric analyses were used to evaluate immunologic parameters. Immunologic parameters were investigated in three patients' groups: not affected by xenobiotic factors (I), not affected by TEL (II), affected by TEL (III).

It was discovered that $CD69^+$-, $CD38^+$-lymphocytes' amount was higher in group III; $CD16^+/56^+$-lymphocytes' amount in the same group was lower. In the same group high IgG to CMV and HVS I level detected as well as crioglobulines presence. In group II myeloperoxidase test was lowered and cation-protein test was raised comparatively to group I.

We conclude that TEL can be a xenobiotic factor increasing membrabe and proliferative immune response, changing neutrophil dependent enzyme processes and reducing antivirus resistence in patients with PSV.

ANTIRIBOSOMAL P ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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We report a case-control study of the occurrence of kidney disease in 17 systemic lupus erythematosus (SLE) patients with anti-ribosomal P antibodies and 17 SLE patients without anti- P antibodies. All patients had renal biopsy. In the group with anti- P antibodies, 5 patients were found to have had liver disease, compared with 2 in the control group, and 17 anti- P positive patients have had kidney disease, compared only one in the control group. We observed 6 SLE patients whose nephrotic episodes were associated with the appearance of antibodies to ribosomal P-protein in the absence of antibodies to native DNA. These statistically significant differences suggest that antibodies to ribosomal P identify a subset of SLE patients at higher risk for kidney and liver involvement. Antibodies to ribosomal P protein in SLE have also been associated with general disease activity and depended of treatment with corticosteroids, immunosuppressors, anticoagulants and intravenous immunoglobulin G.

NATURAL ANTIBODIES TO ENDOGENOUS MEDIATORS IN PATIENTS WITH ARTERIAL HYPERTENSION AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Purpose: To compare the levels of natural autoantibodies (NAA) specifically reacting with endogenous vasoactive mediators in SLE patients suffering arterial hypertension, in patients with essential hypertension and healthy control group.

Methods: We tested sera from 12 SLE pts fulfilling the 1982 ARA criteria for SLE with nephritis and arterial hypertension (I group, 38,1±9,9 SD mean age), 11 pts with essential hypertension (II group, 58,1±5,6 SD mean age) and 9 healthy donors (III group, 40,2±8,4 SD mean age). Solid- phase enzyme immunoassay was used to examine the levels of IgM recognized peptides (bradykinin (BK), angiotensin II (AII) and vasopressin (VP)).

Results: All patients with arterial hypertension showed higher levels of IgM autoantibodies to investigated vasoactive peptides than controls. Mean values IgM antibodies to BK (0,697±0,082 units of OD_{492} in I group and 0,357±0,017 units of OD_{492}) were significantly increased in compare to healthy donors (0,240±0,020 units of OD_{492}, $p<0,001$ in all comparisons). There were decrease the levels of IgM antibodies to AII from SLE group to donors (0,848±0,042, 0,492±0,033 and 0,370±0,016 respectively, $p<0,001$ and $p<0,01$ compared with control). For mean levels of IgM antibodies to VP were observed the same tendency. In I and II groups were obtained higher values IgM to VP than in control (0,908±0,066 and 0,375±0,022 vs 0,290±0,019 in healthy donors; $p<0,001$ and $p<0,01$ respectively).

Conclusions: These data suggest that autoantibodies to endogenous bioregulators take part in the development of arterial hypertension not only in SLE patients, but in patients with essential hypertension. The role of autoantibodies in pathogenesis of these diseases remains not complete clear.

TRH PARTICIPATION ON IMMUNITY REGULATIONS IN MAN

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Interleukin-2 (IL-2) is a pluripotential cytokine which secretion depends on other cytokines and hormonal stimulations. Several reports have revealed the importance of TRH and prolactin (PRL) in regulation of IL-2. The aim of the present study was to determine the in vivo effects of an intravenous bolus of prothyrelin (TRH 0,2 mg i.v.) on interleukin 2 (IL-2) and prolactin (PRL) in 9 healthy women, 8 women with primary hypothyreoidism and 8 women with early rheumatoid arthritis (RA).

IL-2 and PRL responses to TRH (0,2mg i.v.)

	IL-2 (pg/ml)		PRL (ng/ml)	
	0 min	20 min	0 min	20 min
controls n=9	**23,59** ± 15,27	**45,34** ± 18,21	**6,33** ± 1,0	**43,88** ± 9,67
RA n=8	**21,03** ± 12,10	**14,92** ± 7,82	**11,56** ± 6,32	**55,68** ± 20,09
hypothyoidism n=8	**25,46** ± 15,42	**22,62** ± 16,51	**21,69** ± 18,64	**104,91** ± 36,71

The results demonstrate that i.v. bolus of TRH signicantly increased serum levels of IL-2 in control group. Women with primary hypothyroidism have elevated basal levels of IL-2. But after intravenous bolus of TRH the level of IL-2 was decreased. Also striking decline of IL-2 was recorded in women with early rheumatoid arthritis. Basal levels of PRL were different in all groups. The levels of PRL (20 minutes after i.v. TRH) in patients with RA and hypothyroidism were significantly increased comparing to the controls.

Our preliminary results suggest that the level of IL-2 is decreasing and the level of PRL is increasing following TRH, which may be characteristic sign of autoimmune disorders.

We acknowledge financial support by grants from Novartis Slovakia.

SIGNIFICANCE OF GENETIC POLYMORPHISM OF GENE FOR ANGIOTENSIN-CONVERTING ENZYME AND APOE IN PATIENTS WITH PRIMARY GOUT

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Gout is characterised by higher morbidity and mortality due to ischaemic cardiovascular disease caused by serious metabolic conditions and clinical syndroms such as dyslipoproteinemia (DLP), hypertension, nephropathy, diabetes and obesity. DLP is almost invariably present in untreated primary gout (PG) (91,8 % in referred group). Deletion polymorphisms of ACE gene (D/D) is frequently associated with more complicated course of IHD and MI. The aim of the study was to test the presence of possible genetic polymorphisms of IHD candidate genes coding ACE and apoE in patients with PG to elucidate an etiopathogenesis of gouty „complications". We examined 401 patients with puncturally verified gout. In 101 patients also a presence of genetic polymorphisms of apoE (genotypes) and ACE (I/D) was tested by molecular biological methods. A control group consisted of defined healthy probands according to IFCC criteria. All of them have a negative family history as regards IHD, inflammatory and rheumatic diseases. Results: TAG: 4,06 ± 2,05 mmol/l; TC: 5,8 ± 1,3 mmol/l; LDL-C: 3,7 ± 0,8 mmol/l; HDL-C: 0,96 ± 0,23 mmol/l; apoA-I: 1,44 ± 0,4 g/l, apoB: 1,83 ± 0,7 g/l. Polymorphisms: *apoE:* allele frequency ε2 - 0,090 (v. 0,047); ε3 - 0,081 (v.0.862); ε4 - 0,019 (v. 0,09); $P\chi^2$ - 0,01; *ACE:* d/d: 0,323 (v. 0,194); i/d: 0,501 (v. 0,591); i/i: 0,171 (v. 0,215); allele d: 0,573 (v. 0,489); allele i: 0,427 (v.0,511); P χ^2 genotypes < 0,005. Conclusion: We have observed a significantly higher frequency both ε2 and ε4 allele of apoE and D allele of ACE gene polymorphisms. This finding can be clinically important because of higher frequency of hypertension in studied group (49,8 %) and very high frequency of cardiovascular complications in gouty patients.

HAPLOTYPE ANALYSIS IN SLOVAK FAMILIES WITH ALKAPTONURIA

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Alkaptonuria (AKU) is a rare hereditary disorder (1:250 000) caused by deficiency of homogentisate-1,2-dioxygenase (HGO). The disease is characterised by homogentisic aciduria and deposition of oxidative product of homogentisic acid in connective tissues that leads to the painful and disabling arthropathy of the large joints and spine (ochornotic arthropathy). HGO gene maps to chromosome 3q21-23. So far, 34 different disease-causing mutations have been identified within this gene in AKU patients from various populations. Slovakia is a country with significantly increased incidence of AKU (1:19 000). Our recent screening for disease causing mutations in 32 Slovak AKU chromosomes led to identification of 9 different mutations, indicating an unexpected mutation heterogeneity. Here, we present results of haplotype analysis in Slovak AKU families, that provides evidence for the appearance of multiple HGO mutations involving CCC mutational hot spot in Slovakia.

Polymorphisms of angiotensin converting enzyme (ACE), glycoprotein IIIa, and β-fibrinogen genes in neuropsychiatric systemic lupus erythematosus (NP-SLE).

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Etiopathogenetic mechanisms of NP-SLE include brain autoantibodies, cerebral vasculopathy and cytokine-mediated brain inflammation. It has been suggested, that a genetically determined tendency towards development of vascular pathology, thrombus formation and microinfarctions may participate on SLE CNS involvement. With respect to this hypothesis, we analysed the polymorphisms of ACE, glycoprotein IIIa and β-fibrinogen genes, which could contribute to the further compromise of the cerebral circulation. The polymorphisms of ACE and glycoprotein IIIa genes were assigned in 20 patients with NP-SLE fullfilling classification ACR criteria, while the β-fibrinogen gene dimorphism was assessed in 16 patients with NP-SLE. The insertion/deletion (I/D) polymorphism of ACE was ascertained by PCR, the dimorphism of glycoprotein IIIa gene was ascertained by PCR-RFLP with *NciI* digestion and the dimorphism of β-fibrinogen gene also by PCR-RFLP with *HaeIII* digestion. The amplified fragments were separated by electrophoresis and visualized in UV light. As for the ACE gene, the frequency of the D allele was significantly higher in the SLE group (77.5% vs 48.9% in controls, p=0.011). The distribution of the ACE genotype in SLE group was different from that in controls (p=0.026). In case of glycoprotein IIIa gene, the frequency of PI^{A2} allele in the SLE goup was lower than in controls (5% vs 17.6%, p<0.001). Similarly, the glycoprotein IIIa genotype distribution in the SLE group was different from that in controls (p<0.05). As for the β-fibrinogen gene, there was no significant difference in either allele (75.0% vs 76.3%, p=0.76) or genotype distribution (p=0.81) between the NP-SLE and control groups. Our results suggest, that in the multifactorially determined NP-SLE, changes associated with I/D polymorphism could influence vessel wall inflammation. Hypothetically, glycoprotein IIIa could be involved in thrombus formation, intramural deposition of platelet fragments, thus contributing to the thickening and irregularity of small cerebral vessels in NP-SLE. Beta fibrinogen gene does not seem to play a role in NP-SLE.

IL-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM IN PRIMARY SJOGREN´S SYNDROME

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Interleukin-1 receptor antagonist (IL-1Ra) is a naturally occurring counterpart of a proinflammatory and fibrogenic cytokine - interleukin-1. A penta-allelic polymorphism within intron 2 of the IL-1Ra gene (IL-1RN) containing variable numbers of an 86-bp tandem repeat has been described. Allele 2 of the IL-1Ra gene (IL-1RN*2) is considered to be associated with severity of some immunomediated diseases, e.g. definite primary Sjogren´s syndrome and fibrosing alveolitis (FA). Primary Sjogren´s syndrome (SS) is an autoimmune disease of unknown origin with chronic inflammation of exocrine glands and is classified as definite SS, when patients met at least four diagnostic criteria. As other diseases of connective tissue, SS can also lead to development of interstitial lung disease, especially FA. In this study, we have investigated IL-1RN alleles to confirm association of IL-1RN*2 with primary Sjogren´s syndrome and it´s possible relevance to development of secondary lung affection within SS.

Genomic DNA was obtained from 39 patients with definite primary SS and 52 healthy control subjects. All patients and controls were unrelated Slovak Caucasians. IL-1RN alleles were genotyped by PCR-SSP. The alellic and antigen frequencies in both study groups were determined; the data sets were compared using a standard 2 x 2 chi-squared analysis.

The two investigated groups of patients and controls did not differ in the distribution of the IL-1RN alleles. The frequency of IL-1RN*2 in patients with SS (19,2%) and in healthy controls (21,2%) is almost identical. The antigen frequency of IL-1RN*2 in SS is 33,3% , in control subjects 36,5%.

In this Slovak population IL-1RN*2 is not associated with primary Sjogren´s syndrome. These results are contradictory with previously published data about increased frequency of IL-1RN*2 in definite Sjogren´s syndrom in Caucasians of northern European ancestry (Perrier S et al., Clin. Immunol. Immunopatol. 1998, 87: 309-313). This contrast may be explained by different susceptibility markers in populations of different ethnic origin. The conclusion about possible association of IL-1RN*2 and secondary pulmonary involvement in primary SS will be analysed after completing the assessment of pulmonary affection in these patients.

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RELATIONSHIP BETWEN IL-4, IFN-GAMMA AND IMMUNOGLOBULINS IN PRIMARY SJÖGREN'S SYNDROME

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We have analyzed in a group of patients with primary Sjögren's syndrome relation to IL-4, IFN-gamma, CRP and immunoglobulins. Statistical assesment were carried out using Spearman correlations.

The group of patients included of 32 patients (29 women with average age 54.1 y and 3 men average age 58 y). Plasma IL-4, IFN-gamma and C-reactive protein were determined by ELISA. Immunoglobulins (IgA, IgG, IgM) were determined turbidimetric method.

All 32 patients had IL-4 concentrations more than 10 pg/ml and levels IFN-gamma had more than 1 pg/ml. Whereas the number of abnormal CRP, IgA, IgG and IgM results were as fallows: 15.6, 21.9, 31.2, 37.5 %. IL-4 correlated with IFN-gamma (p=0.0003) and IgG correlated with IgA (p=0.0243).

Our results suggest that elevated levels of IL-4 may reflect the intensive activation of T helper type-2 cells which may be responsible for B cell hyperreactivity in this disease.

EFFECT OF CYCLOSPORIN A THERAPY ON THE ACTIVITY OF SERUM ADENOSINE DEAMINASE AND URINARY NEOPTERIN IN PATIENTS WITH SLE

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Both total adenosine deaminase activity (tADA) and neopterin levels are increased in patients with systemic lupus erythematosus (SLE). Results of our previous study (1) on ADA isoenzyme pattern in the serum of SLE patients showed that the origin of the elevated values of tADA activity in these patients was mainly increased ADA2 isoenzyme activity. A common characteristic of ADA2 and neopterin is that both originate from monocyte - macrophage cells.

In this study, we investigated the association between tADA(ADA2) and neopterin in SLE patients (n=42) as well as changes of these parameters in 16 SLE patients undergoing cyclosporin A therapy. The patients received 3.5 mg/kg/day of cyclosporin A.

Urinary neopterin was measured using the HPLC method. tADA was determined by a spectrophotometer using adenosine as the substrate. ADA2 activity was analysed by HPLC after selective inhibition of ADA1 with erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA).

Our result showed significant positive correlation between urinary neopterin and serum tADA activity (r_s=0.65, p<0.0005) in SLE patients. After one week of cyclosporin A therapy, a rapid decrease in neopterin levels was observed in more than half the patients. However, this decrease was usually followed by a moderate increase in neopterin levels after one month. Serum tADA (ADA2) activity also tended to decrease after cyclosporin A therapy, following the changes in neopterin concentration. The decrease in these parameters was reflected in the clinical improvement of patients, evaluated using the ECLAM score. After interrupting the cyclosporin A therapy, neopterin, tADA and ADA2 tended to increase again.

In conclusion, our results show a strong correlation between urinary neopterin and serum tADA(ADA2) in SLE patients. The decrease of neopterin and tADA(ADA2) during cyclosporin A therapy may reflect the immunosuppressive effect of this drug, probably by the indirect suppression of T cell mediated macrophage activation.

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EFFECTS OF RALOXIFENE, A SELECTIVE ESTROGEN RECEPTOR MODULATOR (SERM) ON THYMIC INVOLUTION, T CELL REACTIVITY AND INFLAMMATION

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Raloxifene is a selective estrogen receptor (SERM) approved for prevention of osteoporosis in postmenopausal women.The aim of the following study was to examine if the Raloxifene analogue LY117018 has estrogenic effects on the thymus, T cell responsiveness and on inflammation.Oophorectomised normal mice were treated with sc. injections of equipotent doses of LY and estradiol or vehicle as controls.Effects on thymus involution were studied by analyses of thymus weight, cellularity and histology, while inflammation was determined as T cell mediated hypersensitivity and granulocyte mediated footpad swelling.
LY did not display the anti-inflammatory properties of E2 and only minor effects on thymus involution.The results clearly demonstrate that Raloxifene primarily lacks the immune modulatory effects of E2 on T cell responsiveness and inflammation.

THE EFFECT OF PENICILLAMINE AND AUROTHIOMALATE ON ADENOSINE METABOLISM IN HUMAN LYMPHOCYTES

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Penicillamine and aurothiomalate, depending on the concentration used in medium, included a significant decrease of adenosine deaminase activity and an increase of cyclic adenosine monophosphate level in isolated human lymphocytes. Both drugs decreased also the activity of another degrading enzyme of purine metabolism: purine nucleoside phosphorylase. These data suggest an interference of tested drugs with the metabolism of adenosine which has been to have immunoregulatory properties. Our study concluded that immunosuppressive effect of penicillamine and aurothiomalate in RA patients could be mediated through the interference of the drugs with adenosine metabolism in the lymphocytes.

THE INHIBITORY EFFECT OF ENZYME AND COMBINATION THERAPY WITH CYCLOSPORIN A ON ADJUVANT ARTHRITIS IN RATS

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Recent knowledge on the pathophysiology of rheumatoid arthritis (RA) and drug effect mechanisms have allowed the use of new drugs and drug combinations in RA therapy. This study investigates the efficacy of both enzyme therapy and combined therapy with cyclosporin A in rats with adjuvant arthritis.
Rats with adjuvant-induced arthritis were administered the following drugs: cyclosporin A (2.5 and 5.0 mg/kg/day orally); a mixture of enzymes containing pure substances (bromelain, trypsin, rutin) in the same ratio as in Phlogenzym® (PHL, 45 mg/kg, twice daily intrarectally); and a combination of 2.5 mg cyclosporin A plus 90 mg PHL for a period of 40 days from the adjuvans application. Healthy controls and adjuvant-arthritic rats were also investigated. Levels of serum albumin, swelling and bone erosions (radiographic score) in the hind paws were measured in rats as variables of inflammation and destructive arthritic changes.
Treatment with 5 mg of cyclosporin A and the combination therapy with cyclosporin A plus PHL significantly inhibited both inflammation and destructive arthritis-associated changes. However, 2.5 mg of cyclosporin A or PHL alone inhibited these disease markers, although this was to a lesser extent and at a later stage of arthritis development. A statistically significant decrease in the radiographic score was observed in the groups treated with 2.5 mg cyclosporin A, 5 mg cyclosporin A, and the combination therapy of cyclosporin A plus PHL.
In conclusion, our results show the inhibitory effect of enzyme therapy on adjuvant arthritis in rats, as well as the efficacy of a low dose of cyclosporin A given in combination with enzyme therapy, which may be useful in the treatment of rheumatoid arthritis.

ENDOCRINE CHARACTERIZATION OF MALE LONG EVANS RATS WITH ADJUVANT ARTHRITIS

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Objective: The development of adjuvant arthritis (AA) is controlled by circulating levels of prolactin (PRL) and corticosterone (CORT). To assess the secretory profile of these hormones in Long Evans rats we studied the response of prolactin (PRL) and corticosterone (CORT) to immobilization stress in normal male rats, or 12 hour profile of CORT secretion as well as interleukin-6 (IL-6) expression in adenopituitaries at 6:00 a. m. or 6:00 p. m. in normal and arthritic animals.
Methods: AA was induced by a single injection of cFA at the base of the tail. PRL and CORT were measured by radioimmunoassay. IL-6 expression was measured by dot-blot hybridization with complementary IL-6 cDNA probe.
Results: The rats showed high CORT and PRL response to5 and 20 minute immobilization stress. Arthritic rats displayed activation of CORT but not PRL release. CORT in arthritic rats remained steadily elevated between 6:00 a. m. and 6:00 p. m. which significantly differed from the controls. IL-6 expression was lower in arthritic rats at 6:00 a.m. than in control rats.
Discussion: The of PRL and CORT to stress exposure indicates normal function of PRL and pituitary-adrenocortical axis. The 12 hour CORT secretory profile shows a disturbance of normal daily rhythm in favour of high CORT levels during the day. This may in turn control the expression of IL-6 and thus protect the organism against excessive inflammation.
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SUPPRESSION OF ADJUVANT ARTHRITIS IN RATS BY BOAR SEMINAL IMMUNOSUPPRESSIVE FRACTION

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Objective: The effect of immunosuppressive fraction of boar seminal vesicle fluid (ISF) was tested on the manifestation of adjuvant arthritis in rats.
Methods: ISF inhibitory effect on mitogen-stimulated proliferation was measured in rat splenocytes. Adjuvant arthritis (AA) was induced to male Long Evans rats with Mycobacterium butyricum in adjuvant. ISF was administered repeatedly starting at the time of the induction of arthritis. On day 22 of AA the rats were sacrificed and serum IgG, IgM, albumin, nitrate, corticosterone (CORT) were estimated. In peritoneal macrophages the expression of IL-6 mRNA was measured.
Results: In splenocytes isolated from ISF treated healthy rats the PHA or PWM stimulated proliferative activities were reduced by 47 % and 51 % respectively. Treatment of arthritic rats with ISF attenuated hind paw edema. The production of IgG subclasses dropped in ISF treated AA rats. The reduced thymus mass and albumin levels due to AA was partially restored by ISF. The enhanced serum nitrate and CORT concentrations were attenuated by ISF. The expression of IL-6 was inhibited in arthritic rats after ISF treatment.
Discussion: ISF attenuated the manifestation of AA in rats by lowering the immunoglobulin production probably caused by decreased proliferation of B lymphocytes. The favorable effect of ISF on the manifestation of arthritis suggests its potent use in the model of treatment of the disease.

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SUPPRESSION OF ADJUVANT ARTHRITIS IN RATS WITH CHRONIC BROMOCRIPTINE TREATMENT DOES NOT PREVENT ASSOCIATED OXIDATIVE STRESS

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Objective: To study the effect of prolactin (PRL) inhibition in the early or late phase of adjuvant arthritis (AA) on the development and severity of the disease in male Lewis rats.
Methods: AA was induced by a single injection of cFA at the base of the tail (day 0). PRL was inhibited by chronic administration of dopaminergic agonist bromocriptine (BRC) BRC (5 $mgkg^{-1}$) was injected daily either from day –3 to day 11, (BRC1-AA group), or to day 23 (BRC-AA group) of the disease. On day 24 the rats were sacrificed and serum levels of PRL, corticosterone (CORT), NO^{-}_{2}/NO^{-}_{3}, and oxidative status in the spleen were analyzed
Results: Arthritic rats showed inhibition of PRL secretion but not PRL mRNA expression in adenopituitaries. BRC treatment suppressed PRL release and expression. CORT was stimulated by AA, to the same level in BRC control and BRC-AA groups and further potentiated in BRC1-AA group. Hind paw swelling was reduced though not completely inhibited in BRC1-AA group and totally prevented in BRC-AA group as was the core temperature. Serum levels of nitrate rose in rats with AA remained eleveted in BRC-AA group and was further potentiated in BRC1-AA group. Thiobarbituric acid reactive substances and antioxidant capacity were enhanced in rats with AA and in the same extent in BRC1-AA and BRC-AA groups.
Discussion: These results show a discrepancy between the suppression of clinical symptoms and persisting oxidative stress in AA rats after the PRL inhibition. The potentiation of $NO^{\cdot}$ production after the sudden discontinuation of PRL inhibition during the disease may promote further joint damage.
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INSULIN RESISTANCE IN ADIPOSE TISSUE OF RATS WITH ADJUVANT ARTHRITIS

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Objective: To study the effect of adjuvant arthritis and its suppression with bromocriptine on glucose metabolism in male Lewis rats.
Methods: Adjuvant arthritis (AA) was induced by a single injection of complete Freund's adjuvans (cFA) at the base of the tail (day 0). Bromocriptine (BRC), a dopaminergic agonist, was administred by daily injection (5 $mg.kg^{-1}$) from day -3 to day 23 of the disease. Rats were sacrificed on day 24 and serum albumin, glucose, insulin and insulin binding to fat tissue were determined. In addition, the glucose transport and content of glucose transporter GLUT4 in the same tissue was evaluated.
Results: Arthritic rats (AR) displayed hind paw swelling on the day 23, which was normalised by BRC treatment (controls: 1.28±0.02 ml, AR: 1.78±0.03 ml*, BRC: 1.3±0.05 ml, *statistically significant difference). In paralel the serum albumin was decreased in AR group though not normalised in BRC group (controls: 30.5±0.4 g/l, AR: 23.4±0.4 g/l*, BRC: 26.2±0.4 g/l*). Serum glucose and insulin levels were significantly lower in AR group (4.6±0.2 vs 3.8±0.1 mmol/l* and 11.3±1.5 vs 6.5±0.6 μU/ml*). Despite of above changes the insulin binding to adipose tissue plasma membranes was the same in both control and AR groups. On the other hand, insulin stimulated glucose transport was significantly decreased in isolated fat cells. In accordance with this the content of GLUT4 was lower in AR group and partially normalised by BRC treatment.
Discussion: Our results implay the possible association between inflamatory disease and insulin resistance. However, we can not exclude the effect of undernutrition and muscle inactivity in AR group on glucose metabolism mimicing insulin resistance state.
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