

Int J Immunopathol Pharmacol Vol 22 (4), 2009 Abstracts

1. Autism spectrum disorders and mastocytosis

859-865

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Autism Spectrum Disorders (ASD) are diagnosed in early childhood and include Autism, Asperger's disorder and Pervasive neurodevelopmental disorder - not otherwise specified (PDD-NOS, or atypical autism). ASD are associated with varying degrees of dysfunctional communication and social skills, repetitive and stereotypic behaviors, as well as attention and learning disabilities. Most ASD patients also have food intolerance and other allergic symptomatology indicative of mast cell activation. The number of ASD cases have increased over the last decade to 1/100, but there is no definite pathogenesis or curative therapy. We report that the apparent prevalence of ASD in patients with mastocytosis, a rare disease occurring in 1/4,000 children and characterized by an increased number of hypersensitive mast cells in many organs, is about 1/10 or 10 times higher than the general population. A child with skin mastocytosis (urticaria pigmentosa), and regressive autism is presented to illustrate the point. Allergic, infectious, neuroimmune and environmental triggers may activate mast cells to release vasoactive, inflammatory and neurotoxic molecules. These could disrupt the gut-blood-brain-barriers, and/or activate susceptibility genes, thus contributing to brain inflammation and ASD. *Int J Immunopathol Pharmacol* 2009;22:859-865

2. Clinical evaluation and treatment of acute asthma exacerbations in children

867-878

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This update on treatment of asthma exacerbations in children is the result of an Italian Pediatric Society Task-force, made up of a panel of experts working in 2007-2008. The aim is to give clear indications on the use of the drugs most employed in children, grading the quality of evidence and the strength of recommendations. Suggestions on their limits due to unlicensed and off-label use are reported. The level of evidence and the strength of recommendations for different therapeutic approaches demonstrate that frequently the use of drugs in children is extrapolated from the experience in adults and that more studies are required to endorse the correct use of different drugs in asthmatic children. *Int J Immunopathol Pharmacol* 2009;22:867-878

3. Decrease in phosphorylation of ERK following decreased expression of NK cell-activating receptors in human NK cell line exposed to asbestos

879-888

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YT-CB5, which had been continuously cultured with chrysotile B (CB) asbestos, showed impaired cytotoxicity with decreased expression of NKG2D and 2B4 NK cell-activating receptors. In the present study, the phosphorylation of extracellular signal-regulated kinase (ERK), which is known to induce degranulation downstream of many NK cell-activating receptors, was examined in YT-CB5 by flow cytometry and compared with the control line YT-Org. YT-CB5 exhibited impaired phosphorylation of ERK1/2 induced by the recognition of K562 cells, downstream of a process mediated by Src family kinase and phosphoinositide 3-kinase. YT-CB5 also exhibited impaired phosphorylation of ERK1/2 following incubation with K562 cells in the presence of anti-2B4 antibodies, where co-stimulation by 2B4 augmented the phosphorylation of ERK1/2 in YT-CB5 to a similar degree as in YT-Org. The phosphorylation of ERK1/2 induced by an inhibitor against phosphatase (PP) 1 and PP2A was also lower in YT-CB5 compared with YT-Org. Moreover, bead-bound antibodies to NKG2D, which contribute to cytotoxicity against K562 cells, induced negligible phosphorylation of ERK1/2 in YT-CB5, although antibodies to 2B4 induced a comparatively greater level of phosphorylation. Additionally, peripheral blood (PB-) NK cells with low expression of NKG2D showed lower phosphorylation of ERK1/2 mediated by anti-NKG2D antibodies compared with PB-NK cells with high expression of NKG2D. These results indicate that signal transduction events leading to the phosphorylation of ERK is impaired in YT-CB5 due to decreased expression of NKG2D. Further studies are required to clarify whether this suppressive effect of asbestos exposure on NK cells might promote lung cancer and mesothelioma in people who have inhaled asbestos. *Int J Immunopathol Pharmacol* 2009;22: 879-888

4. Resorcylic acid lactone L-783,277 inhibits the growth of the human adrenal cancer cell line H295R *in vitro*

889-895

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The resorcylic acid lactone L-783,277, isolated from a *Phoma* sp. (ATCC 74403), is a potent and specific inhibitor of MEK (Map kinase kinase) that exerts very interesting pharmacological activities including anti-neoplastic properties. However, the role of this compound in the regulation of endocrine-related cancer cell growth and tumor progression remains unknown. In the present study we have evaluated the effect of L-783,277 on the viability, proliferation and cell cycle of the human adrenocortical carcinoma cell line H295R. L-783,277 inhibited viability (IC₅₀ of 22 μM) and cell proliferation (IC₅₀ of 21 μM) of H295R. At concentrations of 10⁻⁶-10⁻⁸M this effect was associated with the accumulation of H295R cells in S-phase, whereas at concentrations of 10⁻⁹-10⁻¹⁰M a prolonged G1-phase and reduced transition into S-phase were observed. Our findings demonstrate for the first time the anti-proliferative action of L-783,277 on the human adrenocortical H295R cell line. *Int J Immunopathol Pharmacol* 2009;22: 889-895

5. Suppression of MAP kinases inhibits microglial activation and attenuates neuronal cell death induced by alpha-synuclein protofibrils

897-909

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Alpha-Synuclein (alpha-Syn) accounts, as a major component of Lewy bodies (LB), for the filamentous deposits in many cases of neurodegenerative diseases. Yet, little is known about the molecular mechanisms of neuronal loss in these diseases. The correlation between α-Syn oligomerization/aggregation and pathologies raises the key question of which molecular form of α-Syn (i.e. monomeric α-Syn, protofibrils or mature fibrils) represents the damage-inducing culprit in the scenario of synucleinopathies. We show that human α-Syn protofibrils (PFs) are potent activators of parallel proinflammatory signalling pathways (p38 and ERK1/2 MAP kinases and NF-κB) in microglial cells *in vitro*. Furthermore, stereotactic injection of α-Syn PFs into the *substantia nigra* of adult rats leads to a profound activation of microglia and adjacent neuronal cell loss, which can be attenuated by the MAP kinase inhibitor semapimod. We propose that the neurodegenerative process of α-synucleinopathies involves microglial activation through α-Syn released or extruded from cells with pathogenic α-Syn metabolism. Compounds that inhibit the MAPK/NF-κB pathways might be a promising pharmacological strategy for the treatment of the inflammatory component of synucleinopathies including PD. *Int J Immunopathol Pharmacol* 2009;22: 897-909

6. Beta glycosphingolipids suppress rank expression and inhibit natural killer T cell and CD8+ accumulation in alleviating aortic valve calcification

911-918

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CD8 lymphocytes play a role in aortic valve inflammation leading to aortic valve calcification (AVC). RANK is a transmembrane protein that is important in osteoclast differentiation and calcification. beta-glucosylceramide (beta-GC) together with beta-lactosylceramide (beta-LC), the 1:1 combination of beta-glucosylceramide and beta-lactosylceramide, designated "IGL," exerts an immune modulatory effect in various inflammatory disorders in a CD8- and NKT (natural killer T cell)-dependent manner. We hypothesized that IGL may affect the inflammatory condition associated with AVC. AVC was induced in rats by oral administration of a high-adenine, high-phosphorus diet and was assessed by multislice computer tomography. Administration of this diet was associated with a marked increase in CD8 and NKT lymphocyte accumulation in the aortic valve. Administration of IGL led to marked suppression of RANK expression, associated with inhibition of both NKT and CD8 lymphocyte accumulation in the aortic valve. These effects were associated with a significant improvement in the degree of AVC in IGL-treated animals (25 and 53 by Agatston Score, in IGL-treated and controls, respectively). CD8 and NKT lymphocytes play a role in the pathogenesis of AVC, and RANK-mediated NKT inhibition by beta-glycosphingolipids can alleviate AVC. *Int J Immunopathol Pharmacol* 2009;22: 911-918

7. Differential modulation of IgE-dependent activation of human basophils by ambroxol and related secretolytic analogues

919-927

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Ambroxol is a widely used secretolytic agent originally developed from vasicine, a natural alkaloid found in *Adhatoda vasica*, extracts of which have been used to treat bronchitis, asthma, and rheumatism. We previously reported that ambroxol inhibits IgE-dependent mediator secretion from human mast cells and basophils, key effector cells of allergic inflammation. Here, the mechanisms involved in the inhibitory properties of ambroxol were assessed in comparison to other secretolytic analogues (e.g. vasicine, bromhexine, sputolysin). The results show that, in comparison to ambroxol, which reduced IgE-dependent histamine release from basophils at 10 microM-1 mM, the release of the amine was only moderately reduced by sputolysin and vasicine at 1 mM. In contrast, above 10 microM, bromhexine was found to be toxic to basophils *in vitro* as evidenced by induction of histamine release and reduced cell viability. In contrast, the inhibitory actions of ambroxol at concentrations below 1 mM were not toxic and entirely reversible. Ambroxol was also more potent than either sputolysin or vasicine in attenuating basophil IL-4 and IL-13 secretions, whereas bromhexine-induced suppression of *de novo* cytokine synthesis was due to toxic effects. Additionally, ambroxol reduced IgE-dependent p38 MAPK phosphorylation in basophils, unlike bromhexine, sputolysin and vasicine. These results clearly show that ambroxol is both more potent and effective at inhibiting IgE-dependent basophil mediator release and p38 MAPK activity than the

other secretolytic analogues employed. The therapeutic potential of ambroxol as an anti-allergic agent is further underlined by these data. *Int J Immunopathol Pharmacol* 2009;22: 919-927

8. GGTI-2133, an inhibitor of geranylgeranyltransferase, inhibits infiltration of inflammatory cells into airways in mouse experimental asthma

929-935

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Statins have been proposed as a novel treatment of respiratory diseases including asthma. Although the mechanism of anti-inflammatory effect of statins is still unclear, an inhibition of protein prenylation by depleting the downstream metabolites of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase might be involved. To test the hypothesis, the effects of GGTI-2133, a direct inhibitor of geranylgeranyltransferase (GGTase), on antigen-induced airway inflammation were investigated in a murine model of allergic bronchial asthma. Mice were sensitized and repeatedly challenged with ovalbumin antigen (OA). Animals were also treated with GGTI-2133 (5 mg/kg/day, i.p.) once a day before and during the antigen inhalation period. Repeated antigen inhalation caused an infiltration of inflammatory cells, especially eosinophils, into airways. Significant increases in interleukin (IL)-4, IL-13, eotaxin, thymus and activation-regulated chemokine (TARC) and leukotriene B₄ (LTB₄) in bronchoalveolar lavage fluids and total and OA-specific IgE in sera were also found in the antigen-exposed animals. The systemic treatments with GGTI-2133 inhibited the antigen-induced eosinophil infiltration into airways almost completely. However, interestingly, the GGTI-2133 treatment did not affect the levels of these chemotactic factors and IgE. These findings suggest that selective inhibition of GGTase is effective for eosinophilic airway inflammation such as asthma. *Int J Immunopathol Pharmacol* 2009;22: 929-935

9. Effect of PD98059, a selective MAPK3/MAPK1 inhibitor, on acute lung injury in mice

937-950

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The aim of the present study is to evaluate the contribution of mitogen-activated protein kinase 1-3 (MAPK3/MAPK1) in a model of acute lung inflammation in mice. Injection of carrageenan into the pleural cavity of mice elicited an acute inflammatory response characterized by: accumulation of fluid containing a large number of neutrophils (PMNs) in the pleural cavity, infiltration of PMNs in lung tissues and subsequent adhesion molecule expression (I-CAM and P-selectin), lipid peroxidation, and increased production of tumour necrosis factor-alpha, (TNF-alpha) and interleukin-1beta (IL-1beta). Furthermore, carrageenan induced lung apoptosis (Bax and Bcl-2 expression) as well as nitrotyrosine formation, NF-KB activation, and pJNK expression, as determined by immunohistochemical analysis of lung tissues and the degree of lung inflammation and tissue injury (histological score). Administration of PD98059, an

inhibitor of MAPK3/MAPK1 (10 mg/kg) 1 h after carrageenan caused a reduction in all the parameters of inflammation measured. Thus, based on these findings we propose that inhibitors of the MAPK3/MAPK1 signaling pathways, such as PD98059, may be useful in the treatment of various inflammatory diseases.

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10. Effect of phytoncide from trees on human natural killer cell function

951-959

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We previously reported that the forest environment enhanced human natural killer (NK) cell activity, the number of NK cells, and intracellular anti-cancer proteins in lymphocytes, and that the increased NK activity lasted for more than 7 days after trips to forests both in male and female subjects. To explore the factors in the forest environment that activated human NK cells, in the present study we investigate the effect of essential oils from trees on human immune function in twelve healthy male subjects, age 37-60 years, who stayed at an urban hotel for 3 nights from 7.00p.m. to 8.00a.m. Aromatic volatile substances (phytoncides) were produced by vaporizing *Chamaecyparis obtusa* (hinoki cypress) stem oil with a humidifier in the hotel room during the night stay. Blood samples were taken on the last day and urine samples were analysed every day during the stay. NK activity, the percentages of NK and T cells, and granulysin, perforin, granzyme A/B-expressing lymphocytes in blood, and the concentrations of adrenaline and noradrenaline in urine were measured. Similar control measurements were made before the stay on a normal working day. The concentrations of phytoncides in the hotel room air were measured. Phytoncide exposure significantly increased NK activity and the percentages of NK, perforin, granulysin, and granzyme A/B-expressing cells, and significantly decreased the percentage of T cells, and the concentrations of adrenaline and noradrenaline in urine. Phytoncides, such as alpha-pinene and beta-pinene, were detected in the hotel room air. These findings indicate that phytoncide exposure and decreased stress hormone levels may partially contribute to increased NK activity.

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11. TNFalpha and its receptors in psoriatic skin, before and after treatment with etanercept

961-966

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Psoriasis is a chronic inflammatory skin condition characterized by inflammatory dermal infiltrate and hyperproliferative keratinocytes. The pathogenesis of this disease is mediated by a dysregulation of the innate immunity and cytokine production. Tumor Necrosis Factor alpha (TNFalpha) is considered the most important cytokine involved in the pathological mechanism of psoriasis. Recently, several therapies

have been introduced for the treatment of psoriasis that try to block TNF alpha activity. Among these treatments Etanercept is a fusion protein that specifically targets TNF alpha. We performed a study on twelve psoriatic patients aimed at evaluating the effect of Etanercept treatment on the production and expression of TNFalpha and its receptors, in lesional and uninvolved psoriatic skin. We demonstrated that after three month of Etanercept treatment at 50 mg/wk, TNF, TNF-RI and TNF-RII immunostaining in lesional and non-lesional skin samples of patients was greatly reduced, suggesting that this treatment not only acts on stable lesional plaques, but also at a very early stage of the disease. *Int J Immunopathol Pharmacol* 2009;22: 961-966

12. Immunomodulatory activity of a plant extract containing human papillomavirus 16-E7 protein in human monocyte-derived dendritic cells

967-978

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This study reports the immunomodulatory activity on human Monocyte Derived Dendritic Cells (MDDCs) of a vaccine preparation shown to be effective against an HPV16-related tumour in an animal model. The vaccine is composed of extract from *Nicotiana benthamiana* leaves containing HPV16 E7 protein expressed by a potato virus X-derived vector (NbPVX-E7). The effect of the extract was evaluated on MDDC differentiation and maturation by monitoring the phenotypic expression of specific markers. The results show that NbPVX-E7 does not induce monocyte differentiation to dendritic cells, but does induce MDDC maturation. Plant extract does not influence MDDC-uptake of E7-FITC while it significantly improves the Ovalbumin-FITC uptake, considered as a model antigen. Importantly, NbPVX-E7-pulsed MDDCs/PBMCs are able to prime human blood-derived lymphocytes from healthy individuals to induce HPV16 E7-specific cytotoxic activity. This is a propaedeutic study for a possible use of E7-containing plant extract in human immunotherapy of HPV-related lesions. *Int J Immunopathol Pharmacol* 2009;22:967-978.

13. *Moraxella catarrhalis*-specific Th1 cells in BAL fluids of chronic obstructive pulmonary disease patients

979-990

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In chronic obstructive pulmonary disease (COPD) patients' airway mucosa is infiltrated by macrophages and T lymphocytes, potentially reactive to pathogens. We studied the antigen-specificity and the effector functions of *in vivo* activated T lymphocytes isolated from BAL (Bronchoalveolar lavage) of 5 *Moraxella catarrhalis* (Mc)-infected and 5 Mc-non-infected COPD patients. Mc-specific T cells were detected only in BAL or peripheral blood of *Moraxella catarrhalis*-infected patients. The majority of BAL Mc-specific T cells expressed the T helper type 1 (Th1) cytokine profile with high cytotoxic and pro-apoptotic activity. Upon antigen stimulation, all Mc-specific T clones were able to help the immunoglobulin production by autologous B cells and the MMP (Matrix MetalloProteinase)-12 activity by monocytes. Our results suggest a role for Th1-driven response to *Moraxella catarrhalis* in the genesis of COPD. *Int J Immunopathol Pharmacol* 2009;22: 979-990

14. HLA-A*01 is associated with late onset of Alzheimer's disease in Italian patients 991-999

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In this study, the distribution of HLA-A alleles was analyzed in Italian Alzheimer's Disease (AD) patients. Interaction between HLA alleles, APOE genotypes, age of onset, and gender were also analyzed. The results were compared to those obtained in healthy controls (HC). One hundred-seventy-three AD patients and 258 age-and-sex-matched healthy controls were enrolled in the study. AD patients were classified according to age at the onset of disease using quartiles of the distribution. HLA-A genotyping was performed by PCR-SSP; APOE genotyping was performed by RFLP. A correlation between late disease onset and HLA-A*01 was observed. Thus, HLA-A*01, calculated as number of alleles, was significantly more present in patients with age of onset > 74.0 years than in HC (20% vs 10.5%; p=0.014); the distribution of this allele was skewed also in patients 68.1 -74 years of age (16.3%), even if the difference did not reach statistical significance. The relative risk ratio (RRR) of AD onset calculated by a multinomial logistic regression adjusted for sex and presence of APOE-4 confirmed a significant association of HLA-A*01 with AD onset > 74.0 years of age (RRR=2.2; 95%CI: 1.1 - 4.6; p=0.033). A high RRR (2.04) was also present in patients 68.1 - 74 years (p=0.064). Lower age of disease onset did not correlate with HLA-A*01. Data herein suggest that the presence of HLA-A*01 results in delayed AD development, even in patients carrying APOE-4. These results could offer new insights into the etiopathogenesis of Alzheimer's disease. *Int J Immunopathol Pharmacol* 2009;22:991-999

15. Acute rejection features in dual kidney transplant recipients from elderly donors: comparison of calcineurin inhibitor-based and calcineurin inhibitor-free immunosuppressive protocols 1001-1007

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Features of acute rejection in dual kidney transplant have not been studied. The aim of this study is to compare acute rejections in dual kidney transplant recipients from elderly donors on different immunosuppressive protocols. Sixty-nine patients were evaluated: 28 received calcineurin inhibitorbased (group 1) and 41 received calcineurin inhibitor-free immunosuppression (group 2). Histology of all donor kidneys was evaluated before implantation. All rejections showed tubulitis in both groups, and were classified as T-cell mediated acute rejections. Incidence and Banff grade of rejections in the two groups were not significantly different. Late rejections however, were observed in group 1 ($P < 0.01$) whereas steroid-resistant rejections occurred in group 2 ($P < 0.03$). C4d deposition was only observed in group 2. Occurrence of acute rejection was significantly associated with graft loss due to interstitial fibrosis/tubular atrophy in both groups. In group 1 mean serum creatinine levels of patients with rejections at six months and one year were higher than those of patients without rejections ($P < 0.03$ and $P < 0.009$, respectively). In group 2 they were higher at six months ($P < 0.01$) but not at one year. In addition, graft loss due to interstitial fibrosis/tubular atrophy occurred in 3/28 patients in group 1 (10.7%, OR= 1.95, 95%CI 1.02-3.71), and in 1/41 patients in group 2 (2.4%, OR= 0.41, 95%CI 0.07-2.24). Taken together these results suggest better renal function in patients on calcineurin inhibitorfree immunosuppression. In conclusion, acute rejections were detrimental irrespective of the type of immunosuppression, but different features were observed with each therapy. A tailored approach should be advantageous for prevention and treatment of acute rejections. *Int J Immunopathol Pharmacol* 2009;22: 1001-1007.

16. Does spleen volume play a role in patients with HCV-related chronic hepatitis?

1009-1017

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As the lymphotropism of hepatitis C virus (HCV) has already been ascertained, and in the light of the fact that the immune defense system is an organized network composed of functionally interrelated tissues,

this study was carried out to verify the possible involvement of spleen in HCV-related chronic hepatitis. In this cross-sectional study we measured spleen longitudinal diameter by ultrasound, beta2-microglobulin serum levels and splenic artery resistivity index (SARI) by Doppler in 51 patients treated with standard combined (Peg-Interferon plus Ribavirin) antiviral therapy. Thirty-three patients (17 females) completed the regimen and were compared to 31 controls (16 females). The mean basal values of spleen longitudinal diameter were higher in patients with chronic hepatitis than in controls, i.e., 116 mm (9.4) versus 102.7 mm (9.3), $P = 0.0001$. In the same patients a significant trend towards increased spleen longitudinal diameter was found after antiviral therapy, independently of the stage of HCV-related chronic hepatitis. The median values of the beta2-microglobulin concentrations were not significantly higher in the patients with HCV-related chronic hepatitis compared to controls, i.e., 1.3 (0.5 - 2.6) versus 1 (0.6 - 1.4), $P = 0.16$, although during the course of therapy they were significantly increased. SARI values of HCV-related chronic hepatitis patients were different from those of controls, but were unvaried compared to values at the end of treatment. Neither spleen measurements nor serum beta2-microglobulin levels were able to predict therapeutic response to antiviral therapy. A stimulation/expansion of lymphoid tissue was found in patients with HCV-related chronic hepatitis. Such evidence raises the question whether physicians should continue to prescribe antiviral therapy in non-responders and supports the use of a new scheme (SLD plus β 2-MG) to diagnose this ongoing, apparently reversible, but nevertheless dangerous immunologic process. *Int J Immunopathol Pharmacol* 2009;22:1009-1017

17. Microbiological and biochemical effectiveness of an antiseptic gel on the bacterial contamination of the inner space of dental implants: a 3-month human longitudinal study 1019-1026

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Microbial penetration inside the implant's internal cavity produces a bacterial reservoir that is associated with an area of inflamed connective tissue facing the fixture-abutment junction. The aim of this clinical trial is to evaluate the effectiveness of a 1% chlorhexidine gel on the internal bacterial contamination of implants with screw-retained abutments and on the level of AST secreted in periimplant crevicular fluid. Twenty-five patients (aged 29 to 58 years) each received one implant. Three months after the end of the restorative treatment, and immediately after a clinical and radiographic examination and the abutment removal, microbiological samples were obtained from the internal part of each fixture and biochemical samples were collected by peri-implant sulci. The patients were then divided into two groups: the control (CG; n=10) and test (TG; n=15) groups. The CG had the abutment screwed into place and the crown cemented without any further intervention. In contrast, before the abutment placement and screw tightening, the TG had the internal part of the fixture filled with a 1% chlorhexidine gel. Three months later, the same clinical, microbiological and biochemical procedures were repeated in both groups. Total bacterial count, specific pathogens and AST activity were detected. The clinical parameters remained stable throughout the study. From baseline to the 3-month examination, the total bacterial counts underwent a significant reduction only in the TG. In contrast, the AST activity showed a significant increase in the CG. The administration of a 1% chlorhexidine gel appears to be an effective method for the reduction of bacterial colonization of the implant cavity and for safeguarding the health status of peri-implant tissue over a 3-month administration period. *Int J Immunopathol Pharmacol* 2009;22: 1019-1026

18. Exhaled nitric oxide and nitric oxide synthase expression in Hodgkin's disease

1027-1034

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Hodgkin's disease (HD) is a malignant lymphoma with frequent mediastinal involvement, characterized by a significant inflammatory infiltration. Exhaled Nitric Oxide (F_ENO), is present in healthy humans, and has been proven to be increased in eosinophilic diseases such as allergic asthma. We investigated whether F_ENO is increased in mediastinal HD and whether NO is produced by lymphoma tissue. To this aim F_ENO was measured in 56 HD patients, 17 with and 39 without bulky mediastinal involvement, in the period from January 2007 to December 2008. Thirty-seven patients were reassessed after remission. Lymph node biopsies of 10 patients were evaluated for inducible (iNOS) and constitutive (eNOS) nitric oxide synthase expression by immunohistochemistry. F_ENO resulted significantly related to the mediastinal mass maximum diameter (p=0.009) and was significantly higher in patients with as compared to those without bulky mediastinal disease (38.7 ppb, CI_{95%} 19.3-58.0, versus 20.7 ppb, CI_{95%} 16.6-24.7; p=0.009). iNOS and eNOS immunoreactivity was observed in tumour and inflammatory cells (eosinophils and histiocytes). Only in patients with bulky mediastinal HD there was a significant decrease in F_ENO (from 50.4 ppb CI_{95%} 18.0-82.8 to 11.1 ppb CI_{95%} 4.4-17.8, p=0.011). In conclusion, high F_ENO and NOS expression in lymph-nodes indicate that NO is a component of the inflammatory network of HD. F_ENO may be proposed for the assessment and follow up of bulky mediastinal HD patients. *Int J Immunopathol Pharmacol* 2009;22: 1027-1034

19. Endothelial progenitor cells are mobilized after major laparotomic surgery in patients with cancer

1035-1041

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The progression of cancer is largely dependent on neoangiogenesis. Circulating endothelial progenitor cells (EPC) have the ability to form complete vascular structures *in vitro* and play a crucial role in tumor vasculogenesis. Emerging evidence suggests that surgical injury may induce the mobilization of EPC in animal models, and this might have a negative effect on the prognosis of cancer patients. We studied 20 patients (10 men, 65±13 years) undergoing laparotomy for surgical treatment of various forms of abdominal cancer, and 20 age- and sex-matched healthy control subjects. The number of circulating EPC, defined as CD34+/KDR+ cells identified among mononuclear cells isolated from peripheral venous blood, was determined preoperatively and at days 1 and 2 after surgery. Surgery induced a significant increase in circulating EPC levels at day 1 (from 278/mL, interquartile range 171-334, to 558/mL, interquartile range 423-841, p<0.001) and day 2 (709/mL, interquartile range 355-834, p<0.001) compared with baseline values. EPC levels did not change in control subjects. Seven subjects who underwent laparotomic surgery for non-neoplastic disease also showed an increase in EPC levels after

surgery ($p=0.009$ and $p=0.028$ at day 1 and day 2, respectively). We conclude that patients undergoing elective laparotomic surgery for cancer demonstrate an increase in EPC post-operatively. The potential adverse effects of surgical stress-induced EPC mobilization on tumor and metastasis growth in cancer patients need to be addressed in future studies. *Int J Immunopathol Pharmacol* 2009;22: 1035-1041

20. T cell response in acute motor axonal neuropathy 1043-1050

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There is evidence that in the acute axonal motor neuropathy (AMAN) subtype of Guillain-Barré syndrome antibodies to gangliosides, produced through molecular mimicry by antecedent *Campylobacter jejuni* (*C. jejuni*) infection, attack gangliosides expressed in human peripheral nerve axolemma, inducing a primary axonal damage. The aim of this study is to investigate whether the T cell response has a role in AMAN pathogenesis. We isolated monocytes from 4 healthy subjects and 5 AMAN patients with antecedent *C. jejuni* infection and antibodies to GM1 and/or GD1a gangliosides. Immature dendritic cells expressing CD1 molecules cultured with autologous T cells were stimulated with 2 lipopolysaccharides (LPSs) extracted from *C. jejuni* strains containing GM1 and GD1a-like structures and with GM1 and GD1a. The T cell response to LPSs and to gangliosides was determined by measuring the release of IFN- γ and TNF- α . We observed a T cell response to both LPSs in controls and AMAN patients, whereas only AMAN patients showed T cell reactivity to gangliosides GM1 and GD1a with a tight correlation between T cell reactivity to the ganglioside and individual antibody responses to the same ganglioside. T cells responding to gangliosides were CD1c-restricted CD8 positive and CD27 negative. These findings indicate a contribution of cellular immunity in the pathogenesis of AMAN. A possible role for ganglioside-reactive T cells might be to facilitate the production of antibodies against gangliosides. *Int J Immunopathol Pharmacol* 2009;22: 1043-1050

21. Idiopathic recurrent pericarditis refractory to colchicine treatment can reveal Tumor Necrosis Factor receptor-associated periodic syndrome 1051-1058

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Recurrences develop in up to 20-50% of patients with acute pericarditis. Although different causes of recurrent pericarditis have been identified, the etiology remains obscure in most cases which are therefore labelled as idiopathic. Autoinflammatory syndromes include familial Mediterranean fever (FMF), due to mutations in the *MEFV* gene, and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), due to mutations in the *TNFRSF1A* gene. Recurrent pericarditis is a common feature of both conditions, but it rarely occurs alone. Colchicine is the standard treatment for FMF, while patients with TRAPS do not respond to colchicine therapy, but are responsive to corticosteroids. Based on the proven efficacy of colchicine in preventing polyserositis in FMF, colchicine has been proposed for the treatment of recurrent pericarditis and is able to decrease the recurrence rate. Our aim was to investigate the possible involvement of *TNFRSF1A* mutations in a group of patients with idiopathic recurrent pericarditis who were refractory to colchicine treatment. Thirty consecutive patients (17 males, 13 females) diagnosed with idiopathic recurrent pericarditis, who were characterized by a poor response to colchicine treatment, were enrolled in the study. Mutations of the *TNFRSF1A* gene were searched for by amplifying, using polymerase chain reaction (PCR), genomic DNA, and direct sequencing. *TNFRSF1A* mutations were found in 4 of the 30 patients. None of these 4 patients had a family history of recurrent inflammatory syndromes or history of pericarditis. One of the 4 patients had a novel heterozygous deletion ($\Delta Y103-R104$) and three patients carried a heterozygous low-penetrance R92Q mutation. Our data suggest that TRAPS should be kept in mind in the differential diagnosis of recurrent pericarditis, and mutation analysis of the *TNFRSF1A* gene should be considered, in addition to *MEFV* analysis, in patients of Mediterranean origin. A poor response to colchicine treatment and/or a steroid-dependence may be the clue to investigate *TNFRSF1A* mutations in patients with idiopathic recurrent pericarditis. *Int J Immunopathol Pharmacol* 2009;22: 1051-1058

22. Natural killer cell activity decreases in workers occupationally exposed to extremely low frequency magnetic fields exceeding 1 μ T 1059-1066

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In a preliminary study a reduction in Natural Killer (NK) cell activity in peripheral blood lymphocytes (PBL) was observed in a group of workers exposed to levels of Extremely Low Frequency-Magnetic Fields (ELF-MF) exceeding 1 μ T. This study was performed to confirm the results. In 121 workers engaged in various occupational activities, individual ELF-MF exposure was monitored for 2 work shifts. Exposure levels were calculated as Time-Weighted Average (TWA). Subjects were classified as Low exposure (TWA \leq 0.2 μ T), Medium exposure (TWA 0.21-0.99 μ T), or Higher exposure (TWA \geq 1 μ T). In higher exposure workers NK activity proved significantly reduced compared to low exposure, ($p < 0.01$). In medium exposure a reduction was also observed, but the difference was not significant. Multivariate analysis also confirmed the relation between exposure and NK activity. It has been suggested that ELF might affect tumour progression by inducing changes in the immune system: due to the role played by NK activity in host defence against cancer, the interference with the NK cell activity observed in this study is in agreement with this hypothesis. Furthermore, an increased risk for some neurodegenerative disorders has been reported in some epidemiological studies in ELF-MF-exposed workers: changes in NK function were also described in these diseases. Our results, showing the effect on NK activity of exposure exceeding 1 μ T, suggest a possible mechanism for ELF-MF effects. This could open new horizons regarding the adverse long-term effects of these fields. *Int J Immunopathol Pharmacol* 2009;22: 1059-1066

23. Proinflammatory cytokine production in HACAT cells treated by eosin: implications for the topical treatment of psoriasis

1067-1075

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Psoriasis is a multifactorial skin dermatosis characterized in its classical form by erythematous and hyperkeratotic plaques on extensor surfaces of the body, that in most cases can be managed therapeutically by topical agents. Hyperproliferation and a marked inflammation in both epidermis and dermis are thought to be driven by interaction of activated type-1 T lymphocytes and antigen-presenting cells and keratinocytes that release several proinflammatory and immunomodulating molecules. The aim of this study is to investigate whether tetrabromofluorecin, commonly known as eosin, a classical compound traditionally topically used in psoriasis for its presumed anti-inflammatory activities, is able to modulate the production of TNF-alpha, IL-6 and IL-8 that are recognized as the most active and characterized cytokines in the pathogenesis of this skin disorder. HaCaT cell line was used to verify the effects on epidermal inflammation by eosin at scalar doses after testing the viability of cells. Two different population of cells, one stimulated by IFN-gamma and one non-stimulated, were cultivated in presence of tolerable concentrations. The expression and release of IL-6, IL-8, IL10, and TNF-alpha were analysed by RT-PCR and ELISA, respectively. Our results show that tolerable concentrations of eosin were 0.05%, 0.02%, and 0.01%. The expression and production of TNF-alpha, IL-8 and IL-6 were dramatically reduced in presence of eosin 0.05% and 0.02% and the action of eosin was more pronounced on TNF-alpha. In agreement with clinical data, our results show that in presence of tolerable concentrations, eosin seems to influence remarkably the production of three important cytokines involved in the hyperproliferation and inflammatory process, giving a specific explanation of its efficacy and supporting its topical use in the clinical setting. *Int J Immunopathol Pharmacol* 2009;22: 1067-1075

24. Clinical importance of eosinophil count in nasal fluid in patients with allergic and non-allergic rhinitis

1077-1087

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Eosinophil count in nasal fluid (ECNF) was used to differentiate nasal pathologies. Receiver Operating Characteristic (ROC) curve analysis and the area under the curve (AUC) were performed to evaluate the ECNF's accuracy in distinguishing allergic rhinitis (AR) from non-allergic rhinitis (NAR). We also evaluated the accuracy of ECNF in recognizing patients with mild and severe symptoms of rhinitis and patients with ineffective and effective clinical responses to antihistamines. 1,170 consecutive adult patients with a clinical history of rhinitis were studied. ECNF's median in AR was 6.0 and 2.0 in NAR and the best cut-off value was > 3.0, AUC = 0.75. ECNF's median in AR with mild nasal symptoms was 3.0 and 7.0 with severe symptoms, and the best cut-off value was 4.0, AUC = 0.90. ECNF's median in

NAR with mild nasal symptoms was 2.0 and 8.5 with severe symptoms, and the best cut-off value was > 4.0, AUC = 0.86. ECNF's median in AR with effective clinical response to antihistamines was 4.0 and 8.0 with ineffective response, the best cut-off value was ≤ 5.0 , AUC = 0.94. ECNF's median in NAR with an effective clinical response to antihistamines was 1.0 and 2.0 with ineffective response, and the best cut-off value was ≤ 3.0 , AUC = 0.64. Our results suggest an interesting practical use of ECNF data as evaluator of the clinical severity both AR and NAR. As predictor of the clinical response to antihistamines, ECNF is accurate only in patients with AR. The ECNF's performance was moderately accurate in distinguish patients with AR and NAR. *Int J Immunopathol Pharmacol* 2009;22: 1077-1087

25. Sublingual immunotherapy affects specific antibody and TGF-beta serum levels in patients with allergic rhinitis

1089-1096

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Allergic rhinitis (AR) is characterized by Th2 polarized immune response, such as increased IL-4 and reduced IFN-gamma production. Sublingual allergen-specific immunotherapy (SLIT) induces several immunological changes, most of which are still little known. The aim of this study is firstly to investigate the changes of allergen-specific IgE, IgG, IgG4, and IgA serum levels after SLIT. Secondly, this study aims at relating immunoglobulin (Ig) values with some Th1-, Th2-, and Treg-dependent cytokines. Twenty-three patients with pollen-induced AR were enrolled, and they assumed two pre-seasonal SLIT courses for 2 years. Serum allergen-specific IgE, IgG, IgG4 and IgA levels were determined by ELISA method at baseline and after each SLIT course. Serum IL-4, IFN-gamma, IL-10, and TGF-beta levels were also assessed. Allergen-specific IgE, IgG, IgG4, and IgA serum levels significantly increased after SLIT. Serum TGF-beta significantly increased after SLIT. There was a significant correlation between IgA and TGF-beta, both after the first and the second SLIT course. In conclusion, the present study provides the first evidence that pollen SLIT significantly affects Ig production, mainly concerning IgA; and IgA increase is related with TGF-beta production. Moreover, this is the first study that measured Ig classes by using a quantitative method. *Int J Immunopathol Pharmacol* 2009;22: 1089-1096

26. *Verbena officinalis* essential oil and its component citral as apoptotic-inducing agent in chronic lymphocytic leukemia

1097-1104

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We evaluated the pro-apoptotic activity of *Verbena officinalis* essential oil and of its main component citral, on lymphocytes collected from normal blood donors and patients with chronic lymphocytic leukemia (CLL). The number of apoptotic cells was greater in CLL patients than in healthy subjects at all different times of incubation (4, 8 and 24 hours) for samples treated with *Verbena officinalis* essential oil (A) and citral (B) as well vs controls at different concentrations (0.1% and 0.01%). The greater pro-apoptotic ability was showed by both essential oil of *Verbena officinalis* and citral at lower concentrations (after 4 h A 0.1%: 17.8% vs 37.1%; A 0.01%: 15.8% vs 52%; B 0.1%: 18.4% vs 46.4%; B 0.01%: 15.8% vs 54.2%; after 8 h A 0.1%: 23% vs 38%; A 0.01%: 22.2% vs 55%; B 0.1%: 32% vs 42.2%; B 0.01%: 22% vs 54.3%; after 24 h A 0.1%: 5% vs 20.7%; A 0.01%: 25.8% vs 47.2%; B 0.1%: 18.4% vs 46.4%; B 0.01%: 15.8% vs 54.2%). Patients carrying deletion 17p13 (p53 mutation) showed a reduced ability to undergo apoptosis with respect to patients with other genomic aberrations or normal karyotype. The proapoptotic activity of *Verbena officinalis* essential oil and citral is thought to be due to a direct procaspase 3 activation. These data further support evidence that indicate natural compounds as a possible lead structure to develop new therapeutic agents.

Int J Immunopathol Pharmacol 2009;22: 1097-1104

27. Vascular endothelial growth factor and e-nitric oxide synthase-mediated regenerative response occurring upon autologous and heterologous bone grafts

1105-1116

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Bone regeneration procedures allow oral rehabilitation with dental implants also in edentulous ridges with severe bone atrophy. The integration of grafted materials with the host tissue can initiate regenerative, inflammatory and apoptotic response. Since molecular mechanisms exist at the basis of such response, the aim of this work is to investigate, by immunohistochemical analyses, the expression of proteins involved in the graft integration process, in parallel to clinical and histological modifications, occurring on sites treated with extraoral autologous bone graft deriving from the parietal region of the calvaria (eAB), intraoral autologous bone graft deriving from mandibular ramus (iAB) and heterologous bone graft from swine (hB) in human patients. In our study, the immunohistochemical expression of BSP, VEGF, eNOS in eAB samples was significantly higher ($p < 0.05$) compared to values recorded in iAB and hB samples. The inflammatory response, investigated by iNOS expression, was found lower in all autologous samples (eAB and iAB) compared to hB, at statistically significant values. Moreover, the expression of the pro-apoptotic molecule, Bax, resulted significantly lower ($p < 0.05$) in eAB than in iAB and hB samples. These values, together with the low number of apoptotic cells detected in autologous samples, suggest a good regenerative response when extraoral autologous bone graft is used in comparison to the response from the other grafts, and also suggest the use of calvaria graft as a predictable therapeutic procedure for repairing severe bone defects in oral and maxillofacial surgery, not only by clinical and biomechanical criteria, but also from a biomolecular aspect.

Int J Immunopathol Pharmacol 2009;22: 1105-1116

28. Hyperimmunoglobulinaemia in Babinga Pygmies is present from infancy

1117-1120

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Pygmies, a population characterized by short stature, have high immunoglobulin (Ig) concentrations. In this study, we evaluated Ig levels in Cameroon's Babinga Pygmies from infancy to adulthood and the effects of a national health program on these Ig levels. We found that IgG and IgM levels were outside the normal range for Italians of the same age and were comparable to those measured in Babinga Pygmies living in the same region by Siccardi in 1975. In conclusion, the hypergammaglobulinaemia of Babinga Pygmies is already present in infants and is not affected by sanitation improvements, suggesting that it could be partly genetically-determined. *Int J Immunopathol Pharmacol* 2009;22: 1117-1120

29. Bevacizumab-related osteonecrosis of the jaw

1121-1123

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We describe a case of jaw bone necrosis after a lung adenocarcinoma bone metastasis, treated the first time in 2004 by means of pneumonectomy and lymph node ablation. One week after a dental extraction, the patient experienced pain in the mandibular region, in conjunction with alveolar bone exposure. Treatment with amoxicillin and clavulanate every 12 hours for 15 days and 0.2% chlorhexidine rinses was administered and there was a remission of infective complications, but not the closure of the exposed alveolar bone. Only at this time did the patient refer that he was treated with bevacizumab therapy immediately after the extraction. A preventive dental assessment of patients scheduled for bevacizumab therapy should be useful as for the zoledronic acid therapy. Dental surgery procedures for patients during bevacizumab therapy should be carefully evaluated and considered as the last choice, to reduce all possible risks and prevent complications. *Int J Immunopathol Pharmacol* 2009;22: 1121-1123

30. Combination treatment with etanercept and an intensive spa rehabilitation program in active ankylosing spondylitis

1125-1129

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The aim of this study is to determine the effects of a combination treatment with etanercept and spa rehabilitation versus etanercept alone on function, disability and quality of life in a group of patients with active ankylosing spondylitis (AS). Sixty patients with AS underwent etanercept as suggested by ASAS/EULAR recommendations. As the clinical and laboratory conditions improved, 30 patients

accepted the proposal of coupling the medical therapy with a 7-day rehabilitation program in a thermal baths centre; the remaining 30 subjects continued to take the biologic agent alone. The comparisons between the 2 groups were made after 3 and 6 months. The primary outcome was an improvement in BASFI. The secondary outcome was an improvement in the visual analogic scale of EuroQol (EQ-5D_{vas}). After 6 months a statistically significant improvement in BASFI ($p < 0.05$) and EQ-5D_{vas} ($p < 0.05$) scores was observed in both groups. The mean change in EQ-5D_{vas} value showed a statistically significant difference in favour of the combination therapy group versus the monotherapy group (22 vs 32, $p < 0.05$). A therapeutic regimen combining etanercept with an intensive rehabilitation program contributes to disability reduction and ameliorates quality of life for AS patients. *Int J Immunopathol Pharmacol* 2009;22: 1125-1129

31. Long-term tolerability of Etoricoxib in patients with previous reactions to non-steroidal anti-inflammatory drugs

1131-1134

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Non-steroidal anti-inflammatory drugs frequently cause adverse reactions. This retrospective study was based on analysis of the data obtained from interviews conducted with 173 patients, who underwent and tolerated a challenge test with etoricoxib (a selective cyclooxygenase 2 enzyme inhibitor). Only one of 82 patients who were treated with etoricoxib reported reactions. We can conclude that etoricoxib shows a high long-term tolerability in patients with non-steroidal anti-inflammatory drug hypersensitivity.

Int J Immunopathol Pharmacol 2009;22: 1131-1134

32. Life expectancy of women with lupus nephritis now approaches that of the general population

1135-1141

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Immunosuppressive treatment has changed the prognosis of Lupus nephritis over time, but improvement in prognosis is difficult to analyze in different historical periods, and should be better demonstrated in comparison with life expectancy of sex- and age-matched people. Long-term patient and renal survival of 90 patients diagnosed with Lupus nephritis at our center from 1968 to 2001 with a follow-up time of 14±8 years was retrospectively evaluated. Patient and kidney survival significantly increased over time. Multivariate analyses show that risks of patient and renal death decreased by 8% at each year of follow-up, and increased by more than 5 times in patients aged > 30 years at diagnosis. As only 14 patients were men, relative survival as compared to that of the sex- and age-matched general population of the Piedmont Region was calculated for the 76 women. Improvement in the survival of the cohort of women was seen at any time of follow-up: in particular, it was sharply lower in the first period (relative survival at 5, 10 and 15 years = 0.784, 0.665, and 0.620, respectively) and increased in the second (relative survival at 5, 10 and 15 years = 0.939, 0.921, and 0.850, respectively) nearly approaching that expected for the general population, i.e. 0.993, 0.983 and 0.967, respectively. Taken together, our data allow us to draw the conclusion that life expectancy in women with Lupus nephritis has improved over time, paralleling an improved awareness of the disease and a significant increase in steroid pulse therapy as induction/remission phase. Improvement in survival is for the first time demonstrated to cover the gap with life expectancy of the general population for women with Lupus nephritis.

Int J Immunopathol Pharmacol 2009;22: 1135-1141

33. Mixed panniculitis responding to Cyclosporin-A with a 12-month follow-up: a case report 1143-1146

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Panniculitides represent a heterogeneous group of inflammatory diseases involving subcutaneous fat. Subcutaneous fat is normally organized into adipose cells, adipocytes, and septa of connective tissue. The inflammation involving such tissues can be more represented in septa (septal panniculitis) or in lobules (lobular panniculitis) or be equally distributed in both (mixed panniculitis). A bioptical study is necessary in order to discern between different forms. Vascular involvement is also different in such diseases, as it can interest arteries, or veins, or both. Different grades of fat necrosis can also be observed, such as adipocytes without nuclei, lipophagic necrosis, liquefactive fat necrosis, microcystic fat necrosis, ischaemic fat necrosis. Panniculitis can be idiopathic or secondary to other diseases such as systemic sclerosis, rheumatoid arthritis, systemic erithematous lupus and many others. Therapies usually vary on the single patient but the general orientation leads to the use of immunosuppressive drugs such as thalidomide, corticosteroids, cyclosporin-A, hydroxychloroquine and cyclophosphamide. We report a case of a 19-year-old female affected by primary mixed panniculitis, associated with fever and deep

asthenia, that resolved in a few weeks and was maintained with oral cyclosporin-A. *Int J Immunopathol Pharmacol* 2009;22: 1143-1146

34. The economic burden of biological therapy in rheumatoid arthritis in clinical practice: cost-effectiveness analysis of sub-cutaneous anti-TNF α treatment in Italian patients
1147-1152

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Rheumatoid arthritis (RA), with a prevalence of 0.46%, is found in about 272,004 patients in Italy. The socioeconomic cost of rheumatoid arthritis in Italy in 2002 has been estimated at Euro 1,600 million. Costeffectiveness evaluations have been based on the concept that, with treatment, patients will not progress to the next level(s) of disease severity or will take a longer time to progress, thus avoiding or delaying the high costs and low utility associated with more severe disease. Many cost-effective studies have been based on the variation of Health Assessment Questionnaire (HAQ) in clinical trials. The objective of this study is to perform a cost-effective analysis of 86 patients with rheumatoid arthritis in therapy with adalimumab 40 mg every other week and etanercept 50 mg/week for two years in a population of patients observed in clinical practice. The group of patients in therapy with adalimumab had also taken methotrexate, mean dose 12.4 \pm 2.5 mg/week (22 patients) or leflunomide 20 mg/day (16 patients). The group of patients in therapy with etanercept had also taken methotrexate, mean dose 11.7 \pm 2.6 mg/week (24 patients) or leflunomide 20 mg/day (24 patients). Incremental costs and QALYs (quality adjusted life years) gains are calculated compared with baseline, assuming that without biologic treatment patients would remain at the baseline level through the year. Conversion HAQ scores to utility were based on the Bansback algorithm. The results after two years showed: in the group methotrexate+adalimumab the QALY gained was 0.62 \pm 0.15 with a treatment cost of Euro 26,517.62 and a QALY/cost of Euro 42,521.13. In the group methotrexate + etanercept the QALY gained was 0.64 \pm 0.26 with a treatment cost of Euro 25,020.96 and a QALY/cost of Euro 39,171.76. The result of using etanercept in association with methotrexate is costeffectiveness with a QALY gained under the acceptable threshold of Euro 50,000. These are important data for discussion from an economic point of view when we choose a biologic therapy for rheumatoid arthritis in clinical practice.

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