1. Anti-TNF-alpha inhibitors: a new therapeutic approach for inflammatory immune-mediated diseases: an update upon efficacy and adverse events

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The ongoing progresses in the knowledge of the pathogenic mechanisms of various inflammatory or immune-mediated diseases and the availability of innovative biotechnological approaches have lead to the development of new drugs which add to conventional treatments. TNF-alpha inhibitors (Infliximab, Adalimumab and Etanercept) have demonstrated efficacy either as monotherapy or in combination with other anti-inflammatory or disease modifying anti-rheumatic drugs (DMARDs). The efficacy and safety profile of the TNF-alpha inhibitors can be considered, in general, as a class effect. Nevertheless, some differences may exist among the three agents. In this paper, we will briefly review the indications for the use of the three TNF-alpha inhibitors, the pre-treatment considerations and the reported adverse events. Int J Immunopathol Pharmacol 2009;22:557-565

2. Tumor necrosis factor-alpha antagonists: differential clinical effects by different biotechnological molecules

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Inhibitors of tumor necrosis factor–alpha have deeply changed the therapy of several inflammatory human diseases. For instance, clinical management of rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis have profoundly benefited after the introduction of new therapeutic tools, such as antagonist of TNF-alpha molecule. These drugs include etanercept, a soluble TNF-alpha receptor antagonist, three anti-TNF-alpha antibodies, adalimumab, infliximab, golimumab and certolizumab a humanized Fab fragment combined with polyethylene glycol. These compounds efficiently inhibit several TNF-alpha biological- mediated effects, however, they have also shown differential clinical efficacy in several trials from different autoimmune diseases. It is of clinical relevance that non-responders to one of these drugs often positively responded to another. Different mechanisms of action and diversity in pharmacokinetics of these three compounds may partially explain different clinical effects. However, partially diverse pathogenic mechanisms in different diseases also contribute to differential therapeutic responses. Therefore, these apparently homogeneous agents can not be considered equivalent in their clinically efficacy. Differential therapeutic actions of these drugs may be advantageously used in clinical practice and further improve the great potential of individual TNF-alpha inhibitors. Int J Immunopathol Pharmacol 2009;22:567-572.

3. Systemic sclerosis and cancer

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To review recent advances and current controversies on the association between systemic sclerosis (SSc) and cancer, PUBMED was searched from 1966 to the present using the terms: systemic sclerosis, cancer, morphea, sclerotic diseases. Malignancies, mainly in lung and breast, coexist with idiopathic SSc or with SSc-like disorders, but not with localized forms of scleroderma (morphea), with the exception of squamous cell carcinoma in patients with pansclerotic morphea and skin ulcers. The mechanisms connecting SSc and malignancies are unknown. The occurrence of different cancer types with SSc or SSc-like disorders suggest different underlying mechanisms, including altered immune response, common genetic and environmental links, disease-dependent factors, tumor-derived biologic substances and therapies. The process of sclerosis itself may favor cancer in certain sites, and a reaction between T cells and neoantigens formed during irradiation has been suggested to explain the frequent development of morphea after breast irradiation. Radiotherapy, especially when used for breast cancer, may trigger idiopathic SSc or morphea and influence the severity of preexisting SSc, with the consequence that SSc is considered a relative contraindication to breast radiotherapy. In conclusion, cancer and SSc may be associated, but it is still controversial as to whether there is a causal relationship. Continuing interest in these associations, in particular in the different modalities of associations, may help to understand the underlying biological mechanisms and to identify patients at risk. *Int J Immunopathol Pharmacol* 2009;22:573-578.

4. Impairment in cytotoxicity and expression of NK cell-activating receptors on human NK cells following exposure to asbestos fibers

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Asbestos is well-known for its tumorigenic activity, but its effect on anti-tumor immunity remains unclear. Therefore, we prepared a sub-line of YT-A1 human NK cells exposed to chrysotile B (CB) asbestos (YT-CB5) as an *in vitro* model to analyze the effect of asbestos exposure on NK cells, and examined cytotoxicity and expressions of its related molecules. The cytotoxicity of YT-CB5 against K562 cells decreased compared with the original line of YT-A1 (YT-Org). YT-CB5 exhibited significant decreases in expressions of cell surface NKG2D, 2B4 and intracellular granzyme A. YT-CB5 also exhibited a decrease in the 2B4-dependent cytotoxicity. In addition, the degranulations stimulated via cell surface NKG2D and 2B4 also decreased in YT-CB5. Therefore, peripheral blood NK cells in patients with malignant mesothelioma (MM) were examined and compared with healthy volunteers. NK cells in patients with MM also showed decreases in cytotoxicity against K562. Although the expressions of NKG2D and 2B4 did not decrease in NK cells of MM patients, the expression of cell surface NKp46 decreased. To confirm the effect of asbestos exposure on peripheral blood NK cells, PBMCs were cultured under exposure to CB. NK cells in PBMCs exposed to CB *in vitro* showed a significant decrease in the expression of NKp46, whereas NK cells and alter the expression of NK cell-activating receptors including NKG2D, 2B4 and NKp46 and intracellular perforin/granzymes. cells in PBMCs exposed to glass wool did not show such a decrease. These results indicate that exposure to asbestos has the potential to impair the cytotoxicity of NK. *Int J Immunopathol Pharmacol* 2009;22:579-590.
5. A new compound, 1H,8H-pyran-[3,4-c]pyran-1,8-dione, suppresses airway epithelial cell inflammatory responses in a murine model of asthma

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Clinical and experimental studies have established eosinophilia as a sign of allergic disorders. Activation of eosinophils in the airways is believed to cause epithelial tissue injury, contraction of airway smooth muscle and increased bronchial responsiveness. As part of the search for new antiasthmatic agents produced by medicinal plants, the effects of 270 standardized medicinal plant extracts on cytokine-activated A549 human lung epithelial cells were evaluated. After several rounds of activity-guided screening, the new natural compound, 1H,8H-pyran-[3,4-c]pyran-1,8-dione (PPY), was isolated from Vitex rotundifolia L. To elucidate the mechanism by which the anti-asthmatic responses of PPY occurred in vitro, lung epithelial cells (A549 cell) were stimulated with TNF-alpha, IL-4 and IL-1β to induce the expression of chemokines and adhesion molecules involved in eosinophil chemotaxis. PPY treatments reduced the expression of eotaxin, IL-8, IL-16 and VCAM-1 mRNA significantly. Additionally, PPY reduced eotaxin secretion in a dose-dependent manner and significantly inhibited eosinophil migration toward A549 medium. In addition, PPY treatment suppressed the phosphorylation of p65 and ERK1/2, suggesting that it can inhibit the MAPK/NF-κB pathway. To clarify the anti-inflammatory and antiasthmatic effects of PPY in vivo, we examined the influence of PPY on the development of pulmonary eosinophilic inflammation in a murine model of asthma. To accomplish this, mice were sensitized and challenged with ovalbumin (OVA) and then examined for the following typical asthmatic reactions: an increase in the number of eosinophils in BALF; the presence of Th2 cytokines such as IL-4 and IL-5 in the BALF; the presence of allergen-specific IgE in the serum; and a marked influx of inflammatory cells into the lung. Taken together, our results revealed that PPY exerts profound inhibitory effects on the accumulation of eosinophils into the airways while reducing the levels of IL-4, IL-5, and IL-13 in the BALF. Therefore, these results suggest that PPY may be useful as a new therapeutic drug for the treatment of allergic asthma. Int J Immunopathol Pharmacol 2009;22:591-603.

6. Release of palladium from biomechanical prostheses in body fluids can induce or support PD-specific IFN-gamma T cell responses and the clinical setting of a palladium hypersensitivity

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The increased use of Palladium (Pd) for biomedical applications, which has more than doubled in the last ten years, appears to be associated with an increased frequency of adverse reactions to Pd. The aim of this study is to investigate the relationship between the implant of a biomechanical apparatus containing Pd and the setting of a hypersensitivity to Pd by determining the levels of the metal released in biological fluids, assessing the effects of Pd on peripheral blood mononuclear cell (PBMC) cytokine production and exploring the clinical setting of skin sensitization. Of a total of 3,093 subjects examined in 2006, sensitization to Pd alone or in association with nickel (Ni) was observed in 1.6% and 13.03% of the individuals, respectively. Of these, a group of six subjects positive to Pd and negative to Ni at patch testing were selected on the basis of the oral clinical symptoms in order to measure both the levels of Pd in biological fluids and the degradation of the dental prostheses. Specific Pd measurements were carried out on salivary fluid, urine and serum samples by High Resolution Inductively Coupled Plasma-Mass Spectrometry. In addition, the degradation of the dental prostheses was assessed by both a “leaching test” and an analysis of the micro morphology of orthodontic prostheses. The induction of IFN- gamma production by Pd was assessed in PBMC by the ELISpot assay. Skin sensitization to Pd was evaluated by patch testing and clinical examination. Ten healthy subjects were comparatively tested as controls. We found a specific induction of an IFN-gamma response by Pd in PBMC collected from all the subjects positive to Pd at patch testing. On the contrary, control subjects did not show any response to Pd as assessed by IFN- gamma ELISpot assay or by skin testing. Remarkably, the levels of Pd in all biological samples (saliva, sera, urine) were significantly higher in Pd-sensitized patients than in those collected from controls, reaching the highest concentrations in the urine. The leaching studies gave additional evidence that the dental appliances can release measurable levels of Pd in saliva. Oral clinical symptoms in patients with Pd dental prostheses were associated with measurable levels of Pd in the biological fluids, the induction of Pd-specific IFN-gamma responses in PBMC and the clinical evidence of skin sensitization to Pd. These data suggest that dental appliances may represent an active source of Pd in the body, and this, in turn, can favour the clinical setting of a hypersensitivity to this metal. Int J Immunopathol Pharmacol 2009;22:605-614.

7. Influence of the duffy antigen on pharmacokinetics and pharmacodynamics of recombinant monocyte chemoattractant protein (MCP-1, CCL-2) in vivo

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Monocyte chemoattractant protein-1 (MCP-1, CCL-2) binds to the Duffy antigen (DARC) on red blood cells, which act as a sink for several chemokines including MCP-1. In this study it is hypothesized that DARC may alter the pharmacokinetics of infused recombinant human MCP-1 (rhMCP-1). The primary aim of this first in man trial is to compare the pharmacokinetics of rhMCP-1 in Duffy positive and negative individuals. A randomized, double-blinded, placebo-controlled dose escalation trial was conducted on 36 healthy volunteers. Subjects received infusions of 0.02-2.0 µg/kg rhMCP-1 or placebo for one hour. RhMCP-1 displayed linear pharmacokinetics. Duffy negative individuals reached maximal plasma levels significantly earlier, but overall plasma concentration profiles were not altered. RhMCP-1 markedly increased monocyte counts, and estimated EC₅₀ values were 10-fold higher in Duffy positive than in Duffy negative subjects. Increased monocyte counts were associated with decreased surface expression of intercellular adhesion molecule 1 (ICAM-1, CD54). In contrast, neither CCR-2 or CD11b
expression, nor markers of platelet or endothelial activation, inflammation and coagulation were altered. RhMCP-1 is a highly selective chemoattractant for monocytes in humans. The Duffy antigen only minimally alters the pharmacokinetics of rhMCP-1 for doses up to 2 µg/kg. *Int J Immunopathol Pharmacol* 2009;22:615-625.

8. **Expression of basic fibroblast growth factor, its receptors and syndecans in bladder cancer**

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Basic Fibroblast Growth Factor (bFGF) is a heparin-binding cationic protein involved in a variety of pathological conditions including angiogenesis and solid tumour growth. The basic Fibroblast Growth Factor Receptor (FGFR) family comprises at least 4 high affinity tyrosine kinase receptors that require syndecans for their function. Mounting evidence indicates that syndecans, that bind both bFGF and their FGFRs, will act as stimulators, whereas syndecans that only bind bFGF will act as inhibitors of signaling by sequestering the growth factor. Recent findings have highlighted the importance of syndecans in urological cancers. The aim of this study is to investigate the expression of bFGF, its receptors (R1 and R2) and syndecans (1-4) in invasive urothelial carcinoma and normal-looking urothelium by Western blotting, RT-PCR, and immunohistochemistry analyses. Interestingly, bFGF, FGFR1 and FGFR2 protein levels statistically increased in bladder cancer tissues. mRNA of FGFR1 and syndecans (1-4), showed a statistically significant increase while an mRNA increase in the other molecules analysed was not significant. bFGF, its receptors and syndecan immunostaining were mainly present in the urothelium both in normal-looking tissues and urothelial neoplastic cells. In conclusion, our data report that the bFGF, FGFR and syndecan expressions are altered in bladder tumours.

9. **Sequence uniqueness as a molecular signature of HIV-1-derived B-cell epitopes**

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The complex pathophysiology of human immunodeficiency virus (HIV) infection and the relatively high mutation rate of the retrovirus make it challenging to design effective anti-HIV vaccines. Several attempts have been made during the last decades to elucidate the enigmatic immunology of HIV infection and to predict potential immunogenic peptides for active vaccination using bioinformatic analysis methods. The results obtained to date to address this important problem are scarce. In this study, we exploit available HIV databases and analyse previously characterized HIV-encoded linear B-cell epitopes for their amino acid sequence similarity to the human or murine host proteome. We obtained further documentation that the HIV-derived antibody-targeted sequences mostly coincide with peptide areas rarely shared with the host proteins. **In toto**, our past and present data give clear-cut support to the statement that low-similarity to the host proteome is a major mechanism in defining viral peptide immunogenicity and indicate a possible way for inducing effective, high-titer, and non-crossreactive antibodies to be used in anti-HIV vaccine therapy.


10. **Immunogenicity of allo-vesicle carrying ERBB2 tumor antigen for dendritic cell-based anti-tumor immunotherapy**


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Dendritic cells (DCs) are able to orchestrate innate and acquired immunity and can activate and sustain a long-lasting anti-tumor immune response *in vivo* when used as anti-tumor cell therapy. The selection of the antigen and the choice of its formulation are key points in designing anti-cancer DC-based vaccines. Cell released vesicles/exosomes have been shown to transfer antigens, HLAI/peptide complexes and co-stimulatory molecules to recipient cells. In this study we describe the generation of an allogenic microvesicle cell factory in which the expression of a specific tumor antigen was combined to the expression of co-stimulatory and allogeneic molecules. The DG75 lymphoblastoid cell line was selected as microvesicle producer and transfected with ErbB2, as tumor antigen prototype. The shed microvesicles transferred antigenic components to recipient DCs, increasing their immunogenicity. DC pulsing resulted in cross-presentation of ErbB2 both in HLAI and HLAII compartments, and ErbB2-specific CD8+ T cells from cancer patients were activated by DCs pulsed with vesicle-bound ErbB2. The microvesicle cell factory proposed may represent a source of cell free immunogen to be used for DC-based cancer therapy. *Int J Immunopathol Pharmacol 2009;22:647-658.*

11. **Chlamydia pneumoniae** induces T cell apoptosis through glutathione redox imbalance and secretion of TNF-alpha

Chlamydia pneumoniae persistent infection has been implicated in the pathogenesis of several chronic inflammatory diseases including atherosclerosis, and we hypothesized that modulation of the apoptosis of macrophages and/or T cells by C. pneumoniae infection may contribute to the development of such diseases. We therefore evaluated apoptosis, cytokine response, and redox status in human primary T cells and macrophages infected with C. pneumoniae. In addition, cocultures of T cells and macrophages infected with C. pneumoniae were also carried out. Apoptosis, and levels of glutathione (GSH), glutathione disulfide (GSSG), and tumour necrosis factor (TNF)-alpha were measured by flow cytometry, high performance liquid chromatography and enzyme-linked immunosorbent assay. C. pneumoniae induced apoptosis in T cells as well as in co-cultures of T cells and infected macrophages by marked decrease in GSH/GSSG ratio and increased production of TNF-α, respectively. The results demonstrate that interaction of C. pneumoniae with T cells and/or macrophages characterized by interference with redox status, and secretion of tumour necrosis factor TNF-alpha culminates in the induction of T cell apoptosis and survival of infected macrophages. In conclusion, the inappropriate T cell response against C. pneumoniae and survival of infected macrophages could explain the persistence of this intracellular obligate pathogen in the host-organism; it may contribute to the development of chronic inflammatory diseases, although further studies are needed to clarify such a complex mechanism.


12. Interferon-gamma-release assays detect recent tuberculosis re-infection in elderly contacts

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The tuberculin skin test (TST) does not discriminate between recent and remote latent tuberculosis infection (LTBI). This study was carried out to test two interferon-gamma-based blood assays in recent contacts with high prevalence of remote LTBI. We performed a contact tracing investigation in a nursing home for the elderly, where elderly patients were exposed to a case of pulmonary tuberculosis. TST, QuantiFERON-TB Gold (QFT-G) and T-SPOT.TB (TS.TB) were performed 8 weeks after the end of potential exposure. IFN-gamma measurements were recorded and correlation with exposure was evaluated. Twenty-seven (37.5%), 32 (44.4%) and 16 (22.2%) subjects were TST, TS.TB and QFT-G positive, respectively; agreement between TS.TB and QFT-G was good among exposed subjects only
(K=0.915, 0.218 in unexposed, p<0.001). When amounts of IFN-gamma were corrected for the number of producing T cells, specific IFN-gamma production was significantly different between exposed and unexposed individuals (16.75±5.40 vs 2.33±0.71 IFN-gamma IU/1000 SFC, p=0.0001). QFT-G and TS.TB provided discordant results among elderly contacts. Unlike TST, the specific IFN-gamma response might discriminate between recent and long-lasting tuberculosis infection. Int J Immunopathol Pharmacol 2009;22:669-678.

13. Regulation of CREB activation by P38 mitogen activated protein kinase during human primary erythroblast differentiation

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Among the molecular events underlying erythroid differentiation, we analyzed the signalling pathway leading to cAMP response element binding (CREB) nuclear transcription factor activation. Normal donor blood light density cells differentiated to pro-erythroblasts during the proliferative phase (10 days) of the Human Erithroblast Massive Amplification (HEMA) culture, and to orthochromatic erythroblasts, during the differentiative phase (4 additional days) of the culture. Since erythropoietin was present all over the culture, also pro-erythroblasts left in proliferative medium for 14 days continued their maturation without reaching the final steps of differentiation. p38 Mitogen Activated Protein Kinase (p38 MAPK) and CREB maximal activation occurred upon 4 days of differentiation induction, whereas a lower activation was detectable in the cells maintained in parallel in proliferative medium (14 days). Interestingly, when SB203580, a specific p38 MAPK inhibitor, was added to the culture the percentage of differentiated cells decreased along with p38 MAPK and CREB phosphorylation. All in all, our results evidence a role for p38 MAPK in activating CREB metabolic pathway in the events leading to erythroid differentiation. Int J Immunopathol Pharmacol 2009;22:679-688.

14. Simultaneous characterization of phospho-proteins and cell cycle in activated T cell subsets

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Multi-colour flow cytometry is the only technological platform that can analyse the highly complex cellular composition of the immune system in parallel and at single cell resolution. Analysis of the T cell compartment, in particular, requires the simultaneous measurement of multiple markers in order to account for lineage, phenotype and function. Flow cytometry also enables the analysis of
intracellular signalling events. By combining the expression of surface markers, intracellular cytokines, phosphorylated versus unphosphorylated kinases, cell proliferation and DNA profile, mechanistic and kinetic information of subset-specific signalling may be obtained: this has not previously been achieved. Here we present a protocol which permits all of these aspects to be explored simultaneously. By comparing basic procedures previously described we were able to optimise different variables, including the choice of antibody/fluorochrome pairs, permeabilisation, fixation and labelling time, to obtain the best DNA staining of different cell types. We applied this method to study subset-specific signalling related to cytokine production and DNA synthesis in T cells responding to specific antigens.


15. Angiogenic potential of human dental pulp stromal (stem) cells

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Dental pulp is a heterogeneous microenvironment where unipotent progenitor and pluripotent mesenchymal stem cells cohabit. In this study we investigated whether human Dental Pulp Stromal (Stem) Cells (DP-SCs) committed to the angiogenic fate. DP-SCs showed the specific mesenchymal immunophenotypical profile positive for CD29, CD44, CD73, CD105, CD166 and negative for CD14, CD34, CD45, in accordance with that reported for bone marrow-derived SCs. The Oct-4 expression in DP-SCs, evaluated through RT-PCR analysis, increased in relation with the number of the passages in cell culture and decreased after angiogenic induction. In agreement with their multipotency, DP-SCs differentiated toward osteogenic and adipogenic commitments. In angiogenic experiments, differentiation of DP-SCs, through Vascular Endothelial Growth Factor (VEGF) induction, was evaluated by in vitro matrigel assay and by cytometric analysis. Accordingly, endothelial-specific markers like Flt-1 and KDR were basally expressed and they increased after exposure to VEGF together with the occurrence of ICAM-1 and von Willebrand Factor positive cells. In addition, VEGF-induced DP-SCs maintained endothelial cell-like features when cultured in a 3-D fibrin mesh, displaying focal organization into capillary-like structures. The DP-SC angiogenic potential may prove a remarkable tool for novel approaches to developing tissue-engineered vascular grafts which are useful when vascularization of ischemic tissues is required. Int J Immunopathol Pharmacol 2009;22:699-706.

16. Thalidomide prevents formation of multinucleated giant cells (Langhans-type cells) from cultured monocytes: possible pharmaceutical applications for granulomatous disorders


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Thalidomide is an effective drug for chronic inflammatory diseases, but the mechanism underlying its immunomodulatory action remains uncertain. Thalidomide has been reported to clinically improve chronic inflammatory granulomatous disorders. In such disorders, the granulomas consist of epithelioid cells, scattered lymphocytes and multinucleated giant cells (MNGC; Langhans-type cells). The present experimental approach permitted the reproduction of MNGC formation from peripheral blood monocytes and examination of thalidomide’s effect on it. MNGC can be effectively generated from monocytes cultured in the presence of interleukin-4 (IL-4) and macrophage colony-stimulating factor (M-CSF) for 14 days. Thalidomide can inhibit the formation of MNGC in a dose-dependent manner. MNGC formation was partly inhibited by the presence of neutralizing TNF-α antibody in the responses induced by IL-4 and M-CSF. Autocrinal TNF-α production and modulation of cadherin expression to regulate cell adhesion might be involved in this inhibitory action of thalidomide. Our results support thalidomide’s clinical efficacy in the treatment of chronic granulomatous disorders (granulomatosis).


17. Role of the netrin system of repellent factors on synovial fibroblasts in rheumatoid arthritis and osteoarthritis

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Changes in the expression of repellent factors, i.e., Netrins and their receptors, may be responsible for the invasive behavior of the synovial tissue cells in patients with rheumatoid arthritis (RA) and osteoarthritis (OA). This study was carried out to analyze the expression of Netrins and their receptors in synovial cells of patients with RA, OA, and control subjects without synovial inflammation. Quantitative RT-PCR was performed to measure the expression of Netrin-1, -3, -4, Neogenin, DCC, UNC5A-D. The influence of Netrin-1 on synovial fibroblasts (SF) was analyzed by determining proliferation, migration, and their ability to organize collagen. SF expressed all repellent factors of the Netrin family. When comparing SF of healthy donors to patients with RA and OA, a stronger expression of UNC5B (4 fold) and UNC5C (769 fold) in RA and OA was found, whereas expression of the other molecules revealed no significant differences. Treating the SF-cells with recombinant Netrin-1 resulted in inhibition of migration of RA- and OA-SFs whereas control cells were not affected. The stronger expression of UNC5B and UNC5C receptors might contribute to the disordered phenotype of RA- and OA-SFs. Addition of Netrin-1 reduces the migratory ability of SFs, potentially by repulsion, as seen in neuronal cells in embryonic development. Due to its function, Netrin-1 may constitute a novel target in the treatment of OA and RA. Int J Immunopathol Pharmacol 2009;22:715-722.

18. Immunological parameters to define infection progression and therapy response in a well-defined tuberculosis model in mice

To evaluate novel approaches for tuberculosis (TB) diagnostics and treatment, well-validated animal TB models are needed. Especially the emergence and spread of drug resistant TB requires innovative therapy and accurate parameters for monitoring success or failure of therapy. We developed a TB model in BALB/c mice, in which *Mycobacterium tuberculosis* (Mtb) infection was induced through the natural respiratory route, mimicking human TB infection. The lung showed a mild inflammatory infiltrate consisting of granulomas in the first phase of infection, followed by progressive increase of pneumonic lesions resulting in extensive lung consolidation in the chronic phase. Dissemination to the extra-pulmonary sites was observed. The model was validated in terms of therapeutic outcome. The 26-week standard therapy administered in human pharmacokinetic-equivalent doses, resulted in complete elimination of Mtb in all infected organs, without relapse of infection in the post-treatment period. However, a 13-week therapy, simulating patient non-adherence resulted in relapse of infection. In our quest to find biomarkers for monitoring success or failure of therapy, the concentrations of various cytokines in serum and lung, determined by Cytometric Bead Array (CBA), were evaluated in relation to the *in situ* cytokine expression in the lung, assessed by immunohistochemistry. The level of IFN-γ concentration in serum increased with infection progression, and decreased during effective therapy, and as such appeared to be an appropriate immunological parameter for success or failure of therapy. Relapse of infection, after inappropriate therapy, manifested as an increase in the serum IFN-γ concentration. *Int J Immunopathol Pharmacol* 2009;22:723-734.

19. Immunogenicity of Ra1A and its tissue-specific expression in hepatocellular carcinoma

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In order to understand the immunogenicity of a tumor-associated antigen (TAA), Ras family small GTP binding protein (Ra1A) in hepatocellular carcinoma (HCC), antibody responses to Ra1A were evaluated by enzyme-linked immunosorbent assay (ELISA), Western blotting and indirect immuno-fluorescence assay in sera from patients with HCC and sera from normal individuals. Immunohistochemistry (IHC) study with tissue array slides was also performed to analyze protein expression profiles of Ra1A in HCC and control tissues. This study demonstrated that Ra1A had a relative higher frequency of antibody response in HCC (20.1%) compared to liver cirrhosis (3.3%), chronic hepatitis (0%), and normal individuals sera (0%). Ra1A also showed a stepwise increased expression from normal liver tissues (26.7%), liver cirrhosis tissues (45.0%) to HCC tissues (63.3%). Sensitivity and specificity of anti-Ra1A antibody in detection of HCC was 20.1% and 99.3%, respectively. The data suggested that Ra1A might contribute to liver malignant transformation, and could be used as a potential tumor marker in HCC detection. *Int J Immunopathol Pharmacol* 2009;22:735-743.
20. Molecular study of receptor for advanced glycation end product gene promoter and identification of specific HLA haplotypes possibly involved in chronic fatigue syndrome

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The receptor for advanced glycation end product (RAGE) is thought to play an important role in inflammation. Chronic Fatigue Syndrome (CFS) is a long-lasting fatigue that compromises at least 50% of a subject’s daily activities without other known cause. Immune dysfunction has been implicated and an association with a peculiar genetic cytokine profile, predisposing to an immunomodulatory response of inflammatory nature, was found. The aim of this study is to analyse RAGE polymorphisms and HLA-DRB1 alleles in seventy-five Italian CFS patients and 141 controls matched for age, sex and ethnicity. These two groups underwent genomic study for RAGE –374T/A and –429C/T promoter polymorphisms; moreover, 46 patients and 186 controls were typed for HLA-DRB1 at low resolution molecular level. Of these, 31 patients and 99 controls also underwent “high resolution analysis” to define the HLA-DRB1*11 and DRB1*13 alleles. The haplotypes RAGE-374T, DRB1*04; RAGE-374T, DRB1*09; RAGE-374T, DRB1*11; RAGE-374A, DRB1*13; RAGE-429T, DRB1*04 and RAGE-429C, DRB1*11 were significantly more frequent in CFS patients, whereas RAGE-429C, DRB1*07 would seem protective. A significantly lower frequency of DRB1*1104 (5.4% vs 12.9%, p=0.04, OR=0.39) and a significantly higher frequency of HLA-DRB1*1301 (13.0% vs 5.1%, p=0.006, OR=2.79) were found in CFS patients. A synergic effect was observed with RAGE polymorphism. The OR values strengthened in the following cis combinations: RAGE-374A, HLA-DRB1*1104 (OR=0.27) and RAGE-374A, HLADRBI*1301 (OR=6.23). HLA haplotypes rather than single alleles of RAGE or of DRB1 genes seem to be involved in CFS, probably including a subregion of major interest. Int J Immunopathol Pharmacol 2009;22:745-754.

21. Immunohistochemical expression of prostate stem cell antigen in cystoprostatectomies with incidental prostate cancer

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High expression of Prostate Stem Cell Antigen (PSCA) has been shown to be associated with adverse prognostic features in clinically-diagnosed prostate cancer. The aim of this study is to analyze PSCA expression in cystoprostatectomies with incidental prostate carcinoma (PCa). PSCA expression was evaluated immunohistochemically in normal-looking epithelium (NEp), high-grade prostatic
intraepithelial neoplasia (HGPIN) and pT2a Gleason score 6 acinar adenocarcinoma. The evaluation was carried out on 20 cystoprostatectomies (CyPs) with incidental PCa from men with bladder urothelial carcinoma (UC), and 20 radical prostatectomies (RPs) with hormonally untreated PCa from men with clinically detected PCa. Ki-67 was also investigated. The percentages of PSCA positive cells in HGPIN were significantly higher than in NEp (NEp: CyP, mean 2.92% ± standard deviation 6.26%; RP, 3.5% ± 6.46%. HGPIN: CyP, 13.67% ± 12.78%; RP, 14.67% ± 11.34%) (p<0.001). The proportions of positive cells in PCa were greater than in HGPIN (CyP, 20.25% ± 15.96%; RP, 22.58% ± 13.67%) (p<0.001). For Ki-67 labeling, the proportions of positive nuclei in the CyPs significantly increased from NEp through HGPIN to PCa. A similar trend was seen in the RPs. In the CyPs the percentages of PSCA and Ki67 positive cells were lower than in the RPs, the differences between the CyP and RP compartments being not statistically significant. Our findings suggest that PSCA is a marker associated with neoplastic transformation of prostate cells, both in CyPs and RPs. However, there are no significant differences between CyPs with incidental prostate carcinoma and RPs with clinically diagnosed cancer. Int J Immunopathol Pharmacol 2009;22:755-762.

22. N-acetylcysteine infusion improves hepatic perfusion in the early stages of systemic sclerosis

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The aim of our study is to evaluate portal and hepatic hemodynamic changes after N-acetylcysteine infusion in patients with systemic sclerosis. In an open-label study 40 patients with Systemic Sclerosis (SSc) were treated with 15mg/kg/hour intravenous N-acetylcysteine for 5 consecutive hours in a single day. Hepatic flow volume, congestion index, portal flow volume, resistance index and pulse rate index were measured in each subject before and after infusion. In all patients mean hepatic flow volume (HFV) and mean portal flow volume (PFV) values after the five-hour infusion with NAC increased not significantly. In 22 selected patients with active capillaroscopic pattern, modified Rodnan Total Skin Score (mRTSS) <18 and mild-moderate score to vascular domain of disease severity scale (DSS), mean HFV increased significantly when compared with mean HFV of 18 SSc patients with late capillaroscopic pattern, mRTSS >18 and severe-end stage score to vascular domain of DSS. The results of our study demonstrate that NAC is able to increase HFV and total liver perfusion after a single infusion in SSc patients with low disease activity and severity scores. Int J Immunopathol Pharmacol 2009;22:763-772.


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High purity alumina as well as zirconia ceramics have been widely used as orthopaedic implant
Biomaterials and dental devices displaying optimal, but sometimes exclusive, mechanical properties. In order to combine the advantages of alumina and zirconia ceramic materials different types of composites have been developed in which either zirconia is dispersed in an alumina matrix or vice versa. Orthopaedic and dental implant biomaterials are expected to be in contact with living tissues for a long period of time and their long term toxicity must be carefully evaluated. In this study we report the development of a high performance chromia-doped Zirconia Toughened Alumina (ZTA) material which displays promising mechanical properties in terms of hardness, strength and fracture toughness that make it suitable for prosthesis even for small joints. The long-term biocompatibility of this material was also evaluated, mainly in terms of DNA damage, mutagenicity and cancerogenetic potential in mammalian cells. The results obtained suggest that this new ZTA material does not display any longterm carcinogenic effect and it is suitable for biomedical applications from a cancerogenetic point of view. In conclusion, we report the development of a new chromia-doped ZTA material with interesting properties both from a mechanical and a biocompatibility point of view which warrant further studies on its suitability as a candidate biomaterial for orthopaedic implants and dental devices. *Int J Immunopathol Pharmacol* 2009;22:773-779.

### 24. Herpes simplex virus infection and pemphigus

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Pemphigus is a group of autoimmune blistering diseases of skin and/or mucous membranes caused by the presence of antibodies against adhesion molecules on the cell surface of keratinocytes. In genetically predisposed patients, several factors, including drugs, physical agents, neoplasms, hormones, and viruses, notably herpes simplex virus (HSV), have been hypothesized to trigger or exacerbate the disorder. To clarify whether HSV infection represents an aetiopathogenetic factor for pemphigus or a consequence of the immunosuppressive treatment, skin and/or mucosal swabs from 35 patients with pemphigus vulgaris or pemphigus foliaceus were tested for HSV by polymerase chain reaction. Twentythree of these patients were newly diagnosed, while the remaining 12 had had a previous diagnosis and were under treatment with low-dosage oral corticosteroids. Repeat swabs were taken two weeks after starting intensive immunosuppressive therapy in 8 HSV-negative patients. All skin swabs (n = 27) resulted negative for both HSV-1/2, while oral swabs (n = 30) were positive for HSV-1 in 5 out of the 12 patients who were being treated with oral corticosteroids, but in none (n = 19) of the non-treated group (p = 0.0067, χ² test). Five out of the 8 patients with repeat swabs became positive for HSV-1, prompting us to start antiviral therapy. In conclusion, HSV is unlikely to be a triggering factor for pemphigus, but its presence in pemphigus lesions seems to be a frequent and early complication of immunosuppression. *Int J Immunopathol Pharmacol* 2009;22:781-786.

### 25. Abnormal expression of C3ORF9 gene in patients with myelodysplastic syndromes

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Myelodysplastic Syndrome (MDS) cells present genetic instability and dysregulation of the gene C3ORF9, which was isolated from an MDS cDNA library and codes for a putative protein. We studied the expression of C3ORF9 in MDS syndromes to contribute to the understanding of the pathophysiology of MDS. One hundred and thirty-one patients and 35 healthy controls were involved in our study. Bone marrow aspirates and isolated CD34+ cells were used. Gene expression was estimated by quantitative PCR. C3ORF9 was found to be down-regulated in patients with CMML compared to the controls (p<0.01). There was no difference between RARS and the controls (p=0.1), while increased expression was found in RA, RAEB and RAEB-T (p< 0.01 for all). No mutations or polymorphism were detected in our population. CD34+ cells expressed higher levels of C3ORF9 (p<0.01) in patients. The gene expression was correlated to the percentage of + cells in RAEB and RAEB-T (r = 0.64). The altered C3ORF9 expression was possibly due to different gene regulation in these patients and/or to the increased CD34+ cells.


26. *Transcription profile analysis of Vastus lateralis muscle from patients with chronic fatigue syndrome*

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Chronic fatigue syndrome (CFS) is a disabling condition characterized by unexplained chronic fatigue that impairs normal activities. Many body systems are affected and etiology has not yet been identified. In addition to immunological and psychological aspects, skeletal muscle symptoms are prominent in CFS patients. In an effort to establish which pathways might be involved in the onset and development of muscle symptoms, we used global transcriptome analysis to identify genes that were differentially expressed in *vastus lateralis* muscle of female and male CFS patients. We found that the expression of genes that play key roles in mitochondrial function and oxidative balance, including superoxide dismutase 2, were altered, as were genes involved in energy production, muscular trophism and fiber phenotype determination. Importantly, the expression of a gene encoding a component of the nicotinic cholinergic receptor binding site was reduced, suggesting impaired neuromuscular transmission. We argue that these major biological processes could be involved in and/or responsible for the muscle symptoms of CFS. *Int J Immunopathol Pharmacol 2009;22:795-807.*

27. *Immunoglobulin production pattern is allergen-specific in polysensitized patients*

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Allergic rhinitis (AR) is characterized by Th2 polarized immune response, such as increased IL-4 and reduced IFN-γ production, and by a functional defect of T regulatory cells. This impaired immune
response profile influences the pattern of immunoglobulin production in allergic patients. Therefore, the aim of this study is firstly to investigate the allergen-specific IgE, IgG, IgG4, and IgA serum level pattern in polysensitized AR patients with the same skin prick test positivity to some pollen allergens. Secondly, this study aims at relating immunoglobulin (Ig) values with some clinical and immunological parameters. Eighty polysensitized patients with AR were enrolled. Serum allergen-specific IgE, IgG, IgG4, and IgA for mites, Parietaria, grasses, and birch, TGF-β and sHLA-G were determined by the ELISA method. Allergic symptoms and drugs use were also assessed. Allergen-specific IgE, IgG, IgG4, and IgA serum levels were significantly different for each tested allergen (p=0.0001). There was a significant correlation between IgE levels and allergy severity, whereas IgA had an antagonistic behaviour, considering mite-specific immunoglobulins. In conclusion, the present study provides the first evidence that immunoglobulin production pattern depends on the specificity of the allergenic response. *Int J Immunopathol Pharmacol* 2009;22:809-817.

28. Frailty syndrome is associated with altered circulating redox balance and increased markers of oxidative stress

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Frailty Syndrome (FS) is a condition described in aging and characterized by physical vulnerability to stress and lack of physiological reserve. In this study we aim to define whether circulating oxidative stress correlates to frailty in terms of glutathione balance and oxidative protein damage. In 62 elderly outpatients, classified as frail patients according to Fried's criteria, evaluation of reduced Glutathione (GSH), Oxidized Glutathione (GSSG), Tumor Necrosis Factor-α (TNF-α), Malonaldehyde- (MDA) and 4-hydroxy-2,3-nonenal- (HNE) protein plasma adducts were performed. A significant increase in the GSSG was observed in patients with FS when compared to non-frail. No difference was shown in the GSH amount, suggesting a glutathione oxidation more than impairment of the synthesis. TNF-α, MDA- and HNE- adducts, were significantly higher in FS as compared to non-frail patients. A logistic regression model correlating FS with redox balance showed a close relationship between glutathione ratio (OR= 1.8, 95% CI=1.2-2.5) and MDA adducts (OR= 2.8, 95% CI= 1.6-4.7) to frailty. Our findings show an association between oxidative imbalance and Frailty Syndrome. GSSG/GSH ratio and plasma protein adducts strongly predict the frailty conditions and seem to be reliable and easily measurable markers in the context of the multidimensional analysis of elderly patients. *Int J Immunopathol Pharmacol* 2009;22:819-827.

29. Clodronate combined with a surfactant (Tween 20) does not improve osseointegration: a rabbit immunohistomorphometric study

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Biphosphonates are compounds that inhibit bone reabsorption mediated by osteoclasts or the
progression of periodontal disease independent on the host response to pathogenic bacteria that colonize the tooth surface. The use of bisphosphonates in oral implantology is still in the experimental stage. The aim of this study is to evaluate the efficacy of a non-aminobiphosphonate combined with a surfactant to increase the ability of the drug to link to the implant and bone surfaces in the development of osseointegration in rabbits. Smooth titanium implants were devised to be used on rabbit femurs. A topical administration of clodronate combined with the surfactant (Tween 20) at different concentrations was made on the implant surface and in the implant site to increase the bone and implant adhesiveness. Placebo was given to the control group. New Zealand rabbits were used and sacrificed by CO2 after 8 weeks from the implantations. A histologic and histo-morphometric analysis was carried out. Results did not show significant difference between the tests and the placebo groups. Our data are different from other similar studies obtaining statistically significant differences. These differences could depend on the efficacy of the drug used and on the procedure of application of the drug on the implant. This study demonstrates poor efficacy of clodronate applied topically to the implant and implant site during surgery to increase the percentage of osseointegration in the implant. Further studies using different fixation techniques of the drug may be necessary to confirm the present data. 


30. Allergy to nickel: first results on patients administered with an oral hyposensitization therapy

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Nickel sulphate allergy is the most common contact allergy. In fact, nickel sulphate is an ubiquitous element, contained in various objects and food; it occurs in igneous rocks, as a free metal and together with iron, but it is also a component of living organism, mainly vegetables. We carried out a clinical trial of oral hyposensitization therapy with low doses of nickel in a group of 67 patients affected by systemic allergy to this sensitizer element. We obtained good results on consequent tolerance to nickel in treated patients. Int J Immunopathol Pharmacol 2009;22:837-840.

31. Psoriasis induced by Infliximab in a patient suffering from Crohn’s disease

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We describe the case of a 30-year-old female with no family history of psoriasis and suffering from Crohn’s disease successfully treated with infliximab at the dosage of 5 mg/kg. At the 15th week from the start of therapy, the patient developed a palmoplantar pustular psoriasis, which spread to the arms, trunk and scalp with erythematosquamous plaques. Deeming the dermatitis onset due to the anti-TNF-α, we decided to discontinue infliximab, while starting with a topical therapy with emollients and corticosteroids and a systemic therapy with cyclosporine. These treatments achieved a clear improvement of psoriasis after 2 months and a complete regression of skin lesions after 4 months. Several cases have been reported of psoriasis induced by anti-TNF-α, which have shown to exert an effective therapeutic action on this disease. The pathogenic mechanism of such a paradoxical effect has not yet been explained, though a number of hypotheses were proposed, among which one of the most intriguing is that the rapid and strong blockade of TNF-α could result in an enhancement of INF-α activity with consequent induction of psoriasis.
32. Bromhidrosis induced by *sphingomonas paucimobilis*: a case report


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Bromhidrosis is a clinical disorder characterized by excessive or abnormal foul axillary odour due to the interaction of apocrine glands with micro-organisms which causes a serious personal and social handicap for affected people. We present the case of a 50-year-old caucasian female with bromhidrosis. The patient referred that this symptom had begun two months previously. Her past treatments included antibacterial soap, topical antibacterial agents and perfumes, but none of these relieved the patient of the odour. A cultural examination of axillary smear was carried out and it revealed the presence of ciprofloxacin sensible *Sphingomonas paucimobilis*. Therefore the patient was treated with ciprofloxacin and after 1 week the infection resolved completely. *Int J Immunopathol Pharmacol 2009;22:845-848.*

33. Ivabradine use in refractory unstable angina: a case report

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In this report, we describe the clinical results of ivabradine use in a patient with a serious form of unstable angina. For this patient, it was proposed that no other therapeutic, pharmacologic or surgical, option was available. The patient is a 75-year-old woman who presented with repeated episodes of retrosternal chest pain. She notably had a history of type II diabetes mellitus treated by insulin for several years and complicated by diabetic macroangiopathy. ECG tracings recorded during these episodes showed abnormalities of the lateral repolarization phase of ischaemic nature. There was no measured increase in cardiac enzymes. She was transferred to our CCU with a diagnosis of unstable angina. In our CCU, the patient was treated with nitrates, metoprolol, aspirin, clopidogrel and atorvastatin at maximal sustainable doses. Following persistent clinical-instrumental instability, she was subjected to coronary angiography. This study revealed severe multi-vessel coronary artery disease not amenable to surgery or angioplasty revascularization. In addition to the therapy already provided, a beta-blocker (metoprolol 50 mg x 2/die) and diltiazem (30 mg x 2/die) were added despite their potentially dangerous and adverse chronotrophic effects. Despite this treatment, the patient’s heart rate remained high (between 80 and 100 beats/min). This heart rate appeared to be the main driving cause of her anginal symptoms. At this point, the use of ivabradine seemed the only option, even though use would be ‘off-label’ compared to current indications for the drug’s use. We started with a low dose of 2.5 mg/b.i.d. and titrated up to 5 mg b.i.d. As we titrated, we witnessed a gradual reduction in heart rate. A consequent stabilization of her clinical pattern progressed into an almost unexpected asymptomatic state. After about a week of clinical

34. **Beneficial effects of telmisartan in an HIV+ diabetic insulin-dependent patient**

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In HIV-infected patients with metabolic disorders, as in the general population, there is evidence of hypertension requiring pharmacological treatment. The presence of diabetes constitutes a cluster of particularly high cardiovascular risks in patients, both regarding diabetic damage and hypertensive damage. We used telmisartan to manage high blood pressure values in an HIV-positive patient with insulin-dependent diabetes. Surprisingly, insulin therapy had to be suspended because of hypoglycemic fits and treatment with metformin was started. In conclusion, telmisartan was effective and well tolerated for the control of hypertension in this case and improved sensitivity to insulin. There are interesting effects of this drug in HIV-positive diabetic patients. Thus, if further studies confirm these effects, telmisartan may be the anti-hypertensive drug of first choice in HIV-infected subjects on combined antiretroviral therapy affected with diabetes and metabolic disorders. *Int J Immunopathol Pharmacol* 2009;22:853-858.