

## 1. CD25<sup>high</sup> T cells with a prolonged survival inhibit development of diabetes

Pp 767-780

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The goal of this study is to examine a novel hypothesis that the progression of diabetes is partially due to the weakened survival of CD25<sup>high</sup> T cells, and prolonging survival of CD25<sup>high</sup> T cells inhibits the development of diabetes. Since CD28 co-stimulation is essential for the survival of CD4<sup>+</sup>CD25<sup>high</sup> T cells, we determined whether CD28-upregulated translationally controlled tumor protein (TCTP) prolongs the survival of CD4<sup>+</sup>CD25<sup>high</sup> regulatory T cells (Tregs) by a transgenic approach. The TCTP transgene prevents Tregs from undergoing apoptosis induced by interleukin-2 withdrawal-, dexamethasone-, cyclophosphamide-, and anti-Fas treatment *in vitro*. In addition, transgenic Tregs express higher levels of FOXP3 than wild-type counterparts and maintain suppressive activity, suggesting that TCTP promotes Tregs escape from thymic negative selection, and that prolonged survival does not attenuate Treg suppression. Moreover, TCTP transgenic Tregs inhibit the development of autoimmune diabetes due to increased survival of suppressive Tregs and decreased expression of pancreatic TNF- $\alpha$ . Promoting the survival of CD25<sup>high</sup> T cells leads to prolonged survival of Tregs but not activated CD25<sup>+</sup> non-Treg T cells. Thus, we propose a new model of two phase survival for Tregs. Our results suggest that modulation of Treg survival can be developed as a new therapy for autoimmune diseases.

## 2. Human colostrum T lymphocytes and their effector cytokines actively aid the development of the newborn immune system

Pp 781-786

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Colostrum contains soluble and cellular components, the latter mainly T lymphocytes. We expanded *in vitro* colostrum T lymphocytes (CoTL) to evaluate phenotype and capability of cytokine production. We also considered paired cord blood T-lymphocytes (CBTL) representing the newborn "virgin" immune system. CoTL showed memory phenotype while CBTL expressed mainly naïve phenotype. CoTL included a balanced percentage of helper and cytotoxic subsets. We observed higher percentages of IL-2 (p=0.003) and IL-4 (p=0.027) producing cells by helper rather than by cytotoxic T lymphocytes. The greatest percentage of IFN- $\gamma$  producing cells was in cytotoxic cells (p=0.0048), while no difference was found for IL-10. Cord blood samples consisted of a statistically significant greater percentage of helper than cytotoxic cells (p<0.001), with a low percentage of cytokine producing cells, confirming the immaturity of the newborn's immune system. CBTL percentage of IL-2 producing cells was higher for helper than cytotoxic subset (p<0.001). We observed a greater percentage of IFN-gamma (p=0.001), IL-4 (p=0.003) and IL-10 (p<0.001) producing cells by cytotoxic than helper T lymphocytes. CoTL demonstrated to protect the newborn through the mother's previous immune experience and to supply active cytokines, which can help the postnatal development of both T type 1/T type 2 response.

### 3. Is necroptosis a death pathway in aluminum-induced neuroblastoma cell demise?

Pp 787-796

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Besides being an aggravating factor secondary to major physiological alterations in degenerative diseases, aluminum has also been considered as a risk factor in the etiology. Although many *in vivo* and *in vitro* data are in favor of apoptosis and necrosis being involved in Al induced neurodegenerative processes, there is considerable evidence that very complex events may contribute to neural cell death. Necroptosis, a novel cell death pathway, was recently reported to contribute to ischemia brain injury. It is different from, but associated with, apoptosis and necrosis, the two common major pathways of cell demise. In the present study, SH-SY5Y cells were put under stress by Al, a potential degenerative cell death inducer. Nec-1, a specific inhibitor, was used to identify necroptosis. The characteristics observed in Nec-1 and Al treated SH-SY5Y cells showed that necrotic morphological changes were reduced, and a sharp decrease of necrotic rate was detected. Besides, there were Al-induced mitochondria membrane potential decreasing, reactive oxygen species remaining, and autophagosomes declining. The mechanism of Nec-1's effect on cell death may be related to caspases pathways. To our best knowledge, this is the pioneer report on necroptosis in mixed human neural cell death pathways, which might offer a novel therapeutic target for neurodegenerative diseases, and an extended window for neuroprotection.

### 4. CD203c is overexpressed on neoplastic mast cells in systemic mastocytosis and is upregulated upon IgE receptor cross-linking

Pp 797-806

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The ectoenzyme E-NPP3 (CD203c) has recently been identified as a novel activation-linked cell surface antigen on basophils. In the present study, we examined expression of CD203c on normal mast cells (MC) and bone marrow (bm) MC derived from 85 patients with systemic mastocytosis (SM), including cases with indolent SM (ISM, n=72), SM with associated clonal hematologic non-MC-lineage disease (SMAHNMD, n=6), aggressive SM (ASM, n=3), and mast cell leukemia (MCL, n=4). Surface expression of CD203c was analyzed by multicolor flow cytometry. In patients with SM, bm MC expressed significantly higher amounts of CD203c compared to normal bm MC (median MFI in controls: 260 *versus* median MFI in SM: 516, p<0.05). Slightly lower amounts of CD203c were detected on MC in SM-AHNMD and ASM compared to ISM. To demonstrate CD203c expression in MC at the mRNA level, neoplastic MC were highly enriched by cell sorting, and were found to express CD203c mRNA in RT-PCR analysis. Cross-linking of the IgE receptor on MC resulted in a substantial upregulation of CD203c, whereas the KIT-ligand stem cell factor (SCF) showed no significant effects. In conclusion, CD203c is a novel activation-linked surface antigen on MC that is upregulated in response to IgE receptor cross-linking and is overexpressed on neoplastic MC in patients with SM.

## **5. Growth factors and scaffold composition influence properties of tissue engineered human septal cartilage implants in a murine model**

Pp 807-816

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Several surgical disciplines apply cartilage grafts for reconstructive purposes and have to overcome the scarcity of donor sites for this unique tissue. Employing the techniques of tissue engineering, cartilage might be generated in reasonable amounts for clinical purposes. Application of growth factors together with biochemical and biomechanical scaffold properties influence the process of *ex vivo* transplant production. The aims of this study are: 1) to investigate the influence of IGF-1 and TGFbeta-2 on tissue engineered human septal cartilage *in vitro* and *in vivo* after transplantation in nude mice; 2) to analyse the effect of the polydioxanone (PDS) content of the biodegradable Ethisorb E210™ scaffold on the properties of the implanted constructs. Cells were three-dimensionally cultured on biodegradable Ethisorb E210™ (PGA-PLA-copolymer fleeces with polydioxanone (PDS) adhesions), or on E210™ scaffolds with a reduced polydioxanone content. Wet weight (ww), GAG-, and hydroxyprolin-content, as well as the cellularity of the neocartilage constructs were quantitatively evaluated. Additionally, the *in vivo* resorption of the two types of cell carriers was monitored. Addition of growth factors clearly increased the wet weight of the *in vitro* cultured constructs before transplantation. After transplantation, high PDS content improved the *in vivo* stability and macroscopic morphometric appearance of the tissue engineered specimens and led to enhanced deposition of glycosaminoglycans in transplanted constructs. Hydroxyproline content of the implants was not affected by either growth factors or PDS content. These data suggest a role for IGF-1 and TGFbeta-2 in preparative *in vitro* culture of chondrocytes before implantation, while PDS content of the scaffold is important for *in vivo* properties of the implanted material.

## **6. Autophagy induction in T cell-independent acute hepatitis induced by concanavalin A in SCID/NOD mice**

Pp 817-826

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Concanavalin A (Con A) is known to induce acute hepatitis that is mediated by activation of NKT and T-cell and cytokine production in immunocompetent mice. The observation of Con A-induced autophagic cell death of hepatoma cells via a Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 mediated autophagic pathway made us re-evaluate the effect of Con A-induced hepatitis in mice. Con A was administrated intravenously to BABL/c, SCID, or SCID/NOD mice at doses of 20, 30 or 40 mg/kg, respectively, to induce acute hepatitis. The levels of hepatitis and autophagy induction were both analyzed. We found that Con A can induce acute hepatitis in SCID or SCID/NOD mice with a kinetics similar to that of BALB/c, but requiring a higher dose of Con A. No lymphocyte infiltrations were found in SCID or SCID/NOD mice, and the cytokine productions were different. An autophagy with microtubule-associated protein light chain 3-II conversion was demonstrated in the liver post-Con A injection in SCID/NOD mice. Due to the mannose/glucose-specific binding on cell membrane, Con A can induce a T-cell-independent acute hepatitis with autophagy in SCID/NOD mice.

## **7. No association of the NF-KB1 -94INS/DELATTG promoter polymorphism with relapse-free and overall survival in patients with squamous cell carcinomas of the head and neck region**

Pp 827-832

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The transcription factor, nuclear factor- $\kappa$ B (NF- $\kappa$ B) is known to play a major role in immune response, inflammation and, via apoptosis and proliferation, also in oncogenesis. Transcription of NFKB1, which encodes the subunit p50/p105 of NF- $\kappa$ B, seems to be influenced by an insertion/deletion polymorphism in its promoter region. Accordingly, the goal of this study is to investigate whether this polymorphism can serve as a putative prognostic marker in patients with Squamous Cell Carcinomas of the Head and Neck region (HNSCC). The prognostic value of the -94ins/delATTG NFKB1 promoter polymorphism was analyzed in an unselected series of patients treated with curative intent for HNSCC, including all tumor stages with different therapeutical regimens. Genotyping was performed by means of pyrosequencing, using DNA from paraffin-embedded tissue samples from 364 patients with a median follow-up of 61 (2-143) months. The various genotypes were correlated with relapse-free and overall survival, as well as risk, compared to healthy volunteers. The NFKB1 polymorphism was not related to risk of HNSCC. Kaplan-Meier curves revealed no significant association between the -94ins/delATTG alleles and survival or disease progression of patients with HNSCC. In conclusion, the results suggest that the investigated NFKB1 promoter polymorphism has no prognostic impact on risk or clinical course in HNSCC.

## **8. Endomorphin-1 inhibits the activation and the development of a hyporesponsive-like phenotype in lipopolysaccharide- stimulated THP-1 monocytes**

Pp 833-843

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Endomorphin-1 (EM-1) is an endogenous opioid peptide selectively binding to  $\mu$  opioid receptors (MORs). Besides its analgesic effects on the central nervous system (CNS), it has been recently reported that EM-1 can cross the blood-brain barrier (BBB) and diffuse into the blood, behaving as an analgesic/anti-inflammatory molecule on peripheral tissues, thus leading to the hypothesis that it could represent a soluble modulator of immune cell functions. Interestingly, nothing is known about its possible effects on monocytes, the main circulating cell-type involved in those systemic responses, such as fever and septic states, involving the release of high amounts of pyrogenic inflammatory factors. The aim of this work is to evaluate possible EM-1 effects on lipopolisaccharide (LPS)-stimulated THP-1 monocytes in terms of the production of inflammatory mediators and the instauration of a hyporesponsive-like phenotype which is a main feature of systemic inflammatory responses, and on the development of peripheral monocytes to DC. Our data demonstrate for the first time that EM-1 is able to inhibit both LPS-stimulated monocyte activation, in terms of arachidonic acid, PGE<sub>2</sub>, ROI and NO<sub>2</sub> production and instauration of a hyporesponsive phenotype without any macroscopic effect on DC development. These data support the hypothesis that EM-1 could be involved in modulating monocyte functions during systemic inflammatory reactions, also providing new evidence for its eventual clinical application in endotoxic states.

## **9. Alpha-defensin levels in whole saliva of totally edentulous subjects**

Pp 845-849

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Salivary levels of alpha-defensins 1-4 and histatins 1, 3 and 5 were determined in 11 totally edentulous patients, 11 younger healthy adults with normal gingival mucosa (Control group I) and 8 subjects, age-matched with edentulous patients, having a minimum of 25 teeth (Control group II). Whole saliva was treated with trifluoroacetic acid and the acidic soluble fraction analyzed by High Performance Liquid Chromatography-Mass Spectrometry. The area of the extracted ion current peaks was used for peptide quantification. Levels of  $\alpha$ -defensins 1-4, but not of histatins, were significantly lower in totally edentulous patients with respect to both Control group I and Control group II. The two control groups did not show significant differences. The reduced level of oral alpha-defensins, which are mainly of crevicular origin, is most likely due to the absence of the gingival sulcus in the edentulous subjects. The near absence of alpha-defensins might be in part responsible for the higher vulnerability of the oral cavity to oral pathogen infections observed in totally edentulous patients.

#### **10. Tumor necrosis factor- $\alpha$ in airway secretions from cystic fibrosis patients upregulate endothelial adhesion molecules and induce airway epithelial cell apoptosis: implications for cystic fibrosis lung disease**

pp 851-865

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Airway inflammation plays a crucial role in lung damage in cystic fibrosis (CF) and is characterized by a persistent influx of neutrophils into the airways. We hypothesized that the high levels of inflammatory products that accumulate in the microenvironment of the CF lung contribute to induce the persistent neutrophil recruitment and the airway epithelial damage. Thus, we evaluated the *in vitro* effect of sputum sol phase (SSP) from CF patients on a) adhesion molecule expression by human microvascular endothelial cells (HMECs) and b) apoptosis of human bronchial epithelial cells (HBECs), both wild-type and CFTR-defective. SSP was obtained from 7 clinically stable adult CF patients and 8 patients with an acute exacerbation. HMECs and HBECs were cultured in the absence or presence of SSP. Cell adhesion molecule expression was assessed by flow cytometry and cell death by the detection of histone-associated DNA fragments, caspase activation, and cytochrome c release. SSP obtained from CF patients, especially at the time of an acute exacerbation, induced a) an upregulation of endothelial adhesion molecules on cultured HMECs that was associated with an increase of neutrophil adhesion to these cells, and was mediated at least in part by TNF- $\alpha$  and IL-1 and b) apoptosis of airway epithelial cells, mainly activated by TNF- $\alpha$  pathway. These results suggest that the high concentrations of inflammatory mediators in CF airways contribute both to the chronic neutrophil influx and the airway damage, and support the crucial role of early anti-inflammatory treatment in the disease.

#### **11. CCL16 enhances the CD8<sup>+</sup> and CD4<sup>+</sup> T cell reactivity to human HER-2 elicited by dendritic cells loaded with rat ortholog HER-2**

pp. 867-877

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T cells from HLA-A2+ healthy donors were co-cultured with autologous dendritic cells (DC) loaded with apoptotic tumor cells expressing rat *neu*, and were induced to mature by tumor necrosis factor (TNF)alpha and interleukin (IL)-1beta(mDC*neu*) or by the CCL16 chemokine (CCL16/mDC*neu*). Priming by CCL16/mDC*neu* induces a larger population of T cells that express cytoplasmatic interferon (IFN) alpha, TNF gamma, perforin and granzyme B compared to those primed by mDC*neu*. T cells primed by CCL16/mDC*neu* release IFN gamma in response to human HER-2+ cells and kill human HER-2+ target cells more efficiently than those primed by mDC*neu*. Our results show that both the loading of DC with xenogeneic rat *neu* and their maturation by CCL16 are two issues of critical importance for the elicitation of an effective response to human HER-2 in T cells from normal donors.

## **12. Phosphodiesterase type-5 inhibitor and oxidative stress**

pp. 879-889

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Erectile dysfunction (ED) is a common medical condition that affects the sexual life of millions of men worldwide. Numerous physical and psychological factors are involved in normal erectile function, including neurological, vascular, hormonal and cavernous functions. The current therapy for the condition is pharmacological and psychotherapeutic which regulates the erectile function and amplifies the NO-mediated response. The aim of this work is to test the action of three common phosphodiesterase inhibitors: Tadalafil, Sildenafil Citrate and Vardenafil at 0.05 µM on human monocytes, analyzing the expression of iNOS protein and mRNA by Western blot and rt-PCR, and production of NO by conversion of L-(2,3,4,5)-[3H]Arginine to L-(3H) citrulline. We also tested the efficiency of the antioxidant network by spectrophotometer (SOD, CAT, GPx and Gr), under normal conditions and after stimulation with LPS. The results showed an increase in ROS levels, similar for all the molecules with regard to the antioxidant enzymes. In all cases the treatment determines a response to the limited efficiency, arriving at a situation in which phosphodiesterase inhibitors + LPS clearly show oxidative stress.

## **13. The organophosphate paraoxon has no demonstrable effect on the murine immune system following subchronic low dose exposure**

Pp 891-901

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Paraoxon is the bioactive metabolite of the organophosphate pesticide parathion. Desulphuration of parathion by liver enzymes or sunlight results in the formation of paraoxon which inhibits acetylcholine esterase (AChE) activity. In the present study, we analyzed the effect of a 6-week, subchronic treatment with two different daily intraperitoneal doses (30 or 40 nmol) of paraoxon on the immune system of BALB/c mice. At a dose of 30 nmol/day, body weight of treated animals was unchanged compared to the controls. In contrast, the higher dose (40 nmol/day) induced a reduction in body growth, particularly in the first 3 weeks of treatment, peaking at week 2 when the saline group showed a 14.2-fold increase in body weight gain compared to paraoxon-treated animals. Moreover, mice treated with either dose of paraoxon had a >50% reduction in AChE activity during the first 3 weeks of treatment, but by the end of the treatment (week 6), AChE activity returned to normal. With regard to immunological parameters, there was no significant difference in either total spleen weight or in the ratios of various spleen cell populations between control and paraoxon-treated animals. Furthermore, no changes were observed in mitogen-induced cytokine secretion from splenocytes of paraoxon-treated mice. Finally, subchronic exposure to paraoxon did not alter mortality of mice exposed to a bacterial infection with *Salmonella typhimurium*. These data suggest that although subchronic exposure to paraoxon induced a transient inhibition in AChE activity, it had no demonstrable effect on the host immune system.

#### **14. Carbamazepine transbuccal delivery: the histo-morphological features of reconstituted human oral epithelium and buccal porcine mucosae in the transmucosal permeation**

Pp 903-910

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Transbuccal drug delivery is an attractive way of administration since several well-known advantages are provided, especially with respect to peroral management. Carbamazepine (CBZ) is an anticonvulsant which is useful in controlling neuropathic pain, and it is currently administered by peroral route, although its absorption and bioavailability is limited due to various factors. The oral cavity could be an interesting site for transbuccal CBZ delivery due to two properties: slow administration of constant low drug doses and less dose-related side effects. However, in transbuccal absorption a major limitation could be the low permeability of the mucosa which results in low drug bioavailability; thus the aptitude of the drug to penetrate the buccal mucosa has to be assessed by using tissue models resembling human normal mucosa. In our experience, CBZ well permeates mucosal membranes. In order to assess the efficacy of CBZ transbuccal delivery and to verify the reliability of these tissues in permeability testing before and after the passage of CBZ, the histo-morphological features of reconstituted human oral (RHO) epithelium (E) and buccal porcine mucosae were investigated. Significant histological changes due to CBZ passage were observed both in RHO-E and porcine mucosa. The main findings detected in RHO samples were cellular swellings with a signet ring-like appearance, nuclear swelling, prominent nucleoli lined against the nuclear membrane and the presence of keratohyalin granules. The most striking finding regarding porcine buccal mucosa was a cytoplasmic vacuolization, mainly involving the basal layer.

#### **15. Inhibition of semicarbazide-sensitive amine oxidases decreases lymphocyte infiltration in the early phases of rat liver allograft rejection**

Pp 911-920

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Vascular adhesion protein-1 (VAP-1) has been shown to mediate lymphocyte adhesion to endothelia at sites of inflammation *in vitro* and *in vivo*. VAP-1 is also an ectoenzyme with semicarbazide-sensitive amine oxidase (SSAO) activity. In this study we investigated whether inhibition of SSAO influences the inflammatory infiltration in acute rat liver allograft rejection. BN recipients of DA liver allografts were treated with 50 mg/kg/d semicarbazide, an inhibitor of SSAO, or similar volumes of saline. 10 rats/group were followed for graft survival, and 10 rats/group were sacrificed on day 7 post-transplantation for histology and T-lymphocyte isolation. The area percentage of portal inflammatory infiltrates in the grafts was assessed from digital photomicrographs. The proportion of CD4-, CD8- and IL2-receptor positive lymphocytes in the graft was quantified with flow cytometry. On day 7, semicarbazide treatment significantly decreased the inflammatory infiltrate area in the grafts. CD4-, CD8- and IL2-receptor positive cells were equally affected. However, animal survival was not affected. Blockade of the enzymatic activity of VAP-1 has a significant effect on lymphocyte infiltration early in acute liver rejection. Later, activation of other adhesion pathways can by-pass the blockade caused by VAP-inhibition.

#### 16. Acetylsalicylic acid inhibits proliferation of human bone marrow stromal cells and matrix mineralization

Pp 921-928

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Acetylsalicylic acid (ASA) and other non-steroidal anti-inflammatory drugs have been shown to potentially inhibit bone healing and bone formation in both animal and clinical studies. Due to the extensive diffusion of ASA-based long-term therapies, the implications of such a side-effect are of interest in all types of bone surgery, including bone grafting procedures and dental implant placement. In this study, we investigate the effect of ASA at therapeutic concentrations on the proliferation and osteogenic differentiation of human bone marrow stromal cells (BMSCs). Primary cultures of BMSCs were isolated and expanded. Their proliferation in response to ASA 50, 100 and 200 µg/ml was evaluated by MTT assay and 3H-thymidine incorporation. Cell cycle machinery was also investigated by FACS and analysis of inhibitors of cyclin-dependent kinases (CDKIs). ASA inhibited BMSC proliferation and DNA synthesis in a dose-dependent manner down to 60% of control (ASA 200 µg/ml) at 72 h. Cell cycle analysis showed a decrease of BMSCs in the S and G2/M phases with a concomitant accumulation in G0/1 in ASA treated cells. The finding was associated to increased levels of some CDKIs, namely p27Kip1 and p21Cip1, whereas ASA did not affect p16Ink4A level at any of the concentrations employed. The matrix mineralization, that represents the major feature of the osteogenic commitment, was assessed by a specific staining procedure (von Kossa) and by calcium content determination. Both the methods demonstrated an extensive reduction (>90%) of extracellular calcification at 200 µg/ml ASA. On the basis of our results, we can hypothesize that the widely reported inhibition of bone healing by ASA might be sustained both by a direct anti-proliferative effect on BMSCs and by an alteration of the extracellular calcification.

#### 17. Chronic and acute alcohol exposure prevents monocyte-derived dendritic cells from differentiating and maturing

Pp 929-939

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Increasing evidence suggests that alcohol abuse may be linked to adverse immunomodulatory effects on immune responses. Our study was undertaken to clarify the immunological consequences of chronic and acute alcohol exposure on differentiation and maturation of human dendritic cells (DCs). Using immunochemical and cytofluorimetric analysis we determined the phenotype and functions of monocyte-derived DCs from alcoholics and healthy subjects and analyzed their ability to respond to lipopolysaccharide (LPS) in the presence or absence of ethanol (EtOH) exposure. Our results showed that alcoholics' monocytes differentiated to immature DCs with altered phenotype and functions (alc-iDCs). Alc-iDCs showed fewer CD1a<sup>+</sup> cells, weaker CD86 expression and higher HLA-DR expression associated with lower endocytosis and allostimulatory functions than iDCs from healthy subjects (control-iDCs). Despite these impairments, alc-iDCs produced TNF- $\alpha$  and IL-6 in large amounts. LPS stimulation failed to induce full phenotypical and functional alc-iDC maturation. *In vitro* acute EtOH exposure also prevented alc-iDCs and control-iDCs from maturing in response to LPS. T-cell priming experiments showed that EtOH treatment prevented LPS-stimulated control-iDCs from priming and polarizing naïve allogeneic T cells into Th1 cells, thus favouring a predominant Th2 environment. Collectively, our results provide evidence that chronic and acute alcohol exposure prevents DCs from differentiating and maturing in response to a microbial stimulus.

#### **18. Genotyping of different *Pseudomonas aeruginosa* morphotypes arising from the lower respiratory tract of a patient taken to an intensive care unit**

Pp 941-947

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*Pseudomonas aeruginosa* is an opportunistic pathogen and an ubiquitous environmental bacterium. Fifty-seven days after hospitalization, we isolated three distinct *P. aeruginosa* morphotypes (smooth, rough and mucoid) from the lower respiratory tract of a patient admitted to a Cardiology Intensive Care Unit (ICU). Moreover, a group of nine colony variants, arising from the three *P. aeruginosa* isolates growing in laboratory growth media, were also isolated. The resulting 12 isolates were characterised for antibiotic resistance profile and subjected to genotypic analysis by fluorescent-Amplified Fragment Length Polymorphism (f-AFLP) and automated repetitive extragenic palindromic-PCR (rep-PCR) fingerprinting. The three smooth, rough and mucoid morphotypes presented different antibiotic resistance profiles and genotyping analysis showed that they belonged to distinct clones, indicating that at day 57 after the admission the patient was simultaneously colonized by three distinct *P. aeruginosa* isolates. On the other hand, the nine colony variants presented heterogeneous antibiotic resistance profiles and clustered together with the three parental isolates. The understanding of the link between genotype plasticity and antibiotic resistance may contribute to improving our knowledge of this lifethreatening pathogen.

#### **19. Breath markers of oxidative stress and airway inflammation in seasonal allergic rhinitis**

Pp 949-957

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Oxidative stress (OS) is well documented in asthma, but so far little data has been reported in nonasthmatic patients with Seasonal Allergic Rhinitis (SAR). The aim of this study is to investigate the degree of OS and airway inflammation in patients with SAR, with and without concomitant Asthma (SAR +A), using breath markers in exhaled air and in Exhaled Breath Condensate (EBC). In addition, the effects of natural allergen exposure and intranasal steroid treatment on these markers were evaluated. Exhaled NO (eNO) and CO, combined with measurements of 8-Isoprostane (Iso-8), Leukotriene B4 (LTB4) and nitrate/nitrite in EBC, were performed in 23 patients, 11 with SAR and 12 with SAR+A, and 16 healthy subjects. Iso-8 and LTB4 were significantly increased in both groups of patients (median values 43.6 pg/ml and 138.4 pg/ml in SAR group; 38.9 pg/ml, and 164.6 pg/ml in SAR+A group respectively;  $p > 0.05$ ) compared to healthy subjects (18.6 pg/ml and 7.8 pg/ml;  $p < 0.05$ ). Nitrate/nitrite and eNO levels were elevated in both groups compared to controls, but were significantly higher in the SAR+A compared to SAR group (nitrate/nitrite 9  $\mu\text{M}$  and 3.9  $\mu\text{M}$ ;  $p = 0.025$ ; and eNO 18.5 ppb and 12.5 ppb, respectively;  $p > 0.05$ ). Nasal steroids caused significant reduction in LTB4 and 8-isoprostane levels in both groups of patients ( $p < 0.05$ ), while nitrate levels and eNO concentration were little affected by nasal treatment. OS markers were decreased at normal levels out of pollen season. Natural allergen exposure induces OS and airway inflammation, as assessed by measurements of markers in EBC and exhaled air, in patients with SAR who have no clinical signs of lower airway involvement. Besides, intranasal steroid treatment may have a regulatory role in the OS.

## **20. Proteolysis of milk fat globule membrane proteins in preterm milk: a transient phenomenon with a possible biological role?**

Pp 959-967

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Milk fat globule membrane (MFGM) proteins constitute a milk fraction currently of great interest, as they appear to significantly contribute to milk protective role. We investigated these proteins in human preterm colostrum and milk. For the former we found a peculiar 2-DE pattern, with a spot concentration at low molecular weight, which mass spectrometry analysis showed to be fragments belonging to some MFGM proteins with a well-known biological and especially immunological role: lactadherin, membrane-associated lactoferrin, butyrophilin, clusterin and heavy-chain immunoglobulin. Since we were able to rule out protease activity after specimen collection, we hypothesize the localization of the proteolytic enzymes in the alveolar cell membranes of the mammary gland. This mechanism is probably under hormonal control and the unexpected advent of preterm delivery would not allow hormonal conditions typical of lactation to occur immediately, causing a delay in enzymatic inhibition. This hypothesis is supported by some of our results, picturing a peculiar transient phenomenon of adaptation of the mammary-gland-membrane proteins after preterm delivery. Further studies will be required to verify whether the presence of protein fragments exerts a specific biological and immuno-defensive role in preterm infants, thus adding evidence to the outstanding biological role and benefits of mother's own milk in feeding preterm infants.

## **21. Early cytokine modulation after the rapid induction phase of sublingual immunotherapy with mite monomeric allergoids**

Pp 969-976

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The influence of different treatment schedules of sublingual immunotherapy (SLIT) in activating IL-10-producing T-cells, crucial in inducing allergen-specific tolerance, is not completely understood. The present work was designed to evaluate allergen driven interleukin release by mononuclear cells in the early phase of SLIT, after application of different induction schemes. Twenty mite-allergic patients were enrolled, 10 (group A) treated with a traditional 98 day induction scheme and 10 (group B) with a 16 day scheme with monomeric allergoid vaccine. At the end of the induction phase, the cumulative doses taken by group A and group B patients were equivalent to 50.5 and 50.3 µg of mite group 1 allergens, respectively. The release of Th1-, Th2- and Treg-related interleukins was assessed in culture supernatants of 5 µg/ml Der-p1-stimulated mononuclear cells, isolated before and after the induction phases. No relevant treatment-related side effects were observed. Interleukin release was similar in the two groups at the enrolment. Non-stimulated and Der p 1 stimulated release of studied cytokines was similar in the two groups at enrolment. Der p 1 stimulation significantly increased IL-10 release ( $p < 0.0002$ ) after treatment in group B patients, and this effect was higher ( $p = 0.05$ ) compared to group A patients. Furthermore, at the end of SLIT induction TNF-alpha, IL-4 and IFN-gamma production were reduced in group B patients ( $p < 0.05$ ,  $p = 0.062$  and  $p = 0.060$ , respectively). The rapid induction scheme of sublingual immunotherapy induces an early immune suppression more effectively than the slower one. The rapid induction scheme should be the preferential way to start sublingual immunotherapy, particularly when monomeric allergoids are utilized.

## **22. Hospitalizations for pediatric anaphylaxis**

Pp 977-983

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The aim of the study is to examine the epidemiology of anaphylaxis in hospitalized children in Lazio (Central Italy) and to evaluate the incidence and case fatality rate. We also verified the concordance of diagnosis between the Emergency Department and Ordinary Hospitalizations. In order to obtain these results, we reviewed all ICD-9 codes indicative of anaphylaxis in all primary and secondary diagnoses from 2000 to 2003 in all Emergency Departments, Ordinary Hospitalizations and Day Hospitals in Lazio. We then identified 203 ICD-9 diagnoses of anaphylaxis in children aged between 0 and 17 years. Anaphylactic shock (995.0) accounted for 109 (53.7%) of cases. Food anaphylaxis (995.60 onwards) accounted for 87 (43.0%) of cases. Food anaphylaxis was more frequent in the first years of life. In fact, it decreased from 12.5/100,000 resident children/year in the first year of life to 6.1/100,000 resident children/year in the first two years of life, and less than 3/100,000 resident children/year after the seventh year ( $p < 0.001$ ). Only 12.5% of cases of anaphylaxis diagnosed in Ordinary Hospitalizations were subsequently diagnosed by the Emergency Department as anaphylaxis. Moreover, only 42.3% of the diagnoses of anaphylaxis made in the Emergency Department were later confirmed during Ordinary Hospitalization. In the four years of study, one child died from anaphylaxis. Thus, mortality was 0.038 cases/100,000 resident children/year. In conclusion, the incidence of hospitalization was highest in the first years of life, during which food anaphylaxis accounted for most hospitalizations. The inconsistency of diagnoses between Emergency Departments and Ordinary Hospitalizations suggests the need to

increase awareness of anaphylaxis among health workers.

**23. Investigation on the possible relationship existing between the HLA-DR gene and attention deficit hyperactivity disorder and/or mental retardation**

Pp 985-991

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This study examines the possible relationship existing between the HLA-DR gene and attention deficit hyperactivity disorder (ADHD) and/or mental retardation (MR). The diagnosis of ADHD and mental retardation were established through clinical interviews with the parents, children and teachers, according to the criteria in DSM-IV. HLA-DRB1 genotyping was performed both by polymerase chain reaction–sequence specific primers (PCR-SSP) and by sequence based typing (SBT) in a cohort of 81 affected children and a sample of 100 healthy controls. Here, we report a positive association of HLADR4 with ADHD but not with MR. The study adds confirmation to the role of the HLA-DRB1 in the etiology of some types of childhood neuropsychiatric illnesses.

**24. Antibacterial activity and anti-biofilm effect of chitosan against strains of *streptococcus mutans* isolated in dental plaque**

Pp 993-997

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*Streptococcus mutans* is the major cause of dental plaque and is often associated with biofilm formation. The aim of this study is to evaluate the activity of a hydrosoluble derivative of chitosan against *S. mutans* biofilms *in vitro* and *in vivo*. Strains of *S. mutans* were isolated from the dental plaque of 84 patients enrolled in the study. The antibacterial activity of chitosan was determined by broth microdilutions. The effect of chitosan at different concentrations and exposure times on *S. mutans* biofilms at different phases of development was assessed by a clinical study using the classical “4-day plaque regrowth” experiment in adult volunteers. The MIC values of chitosan were between 0.5 and 2 g/L. Compared to distilled water, the chitosan solution significantly decreased the vitality of plaque microflora ( $p \leq 0.05$ ). Chlorhexidine, used as a positive control, reduced vitality even further. The results showed that *S. mutans* in the adhesion phase (4 h) was completely inhibited by chitosan at any concentration (0.1, 0.2, 0.5X MIC) or exposure time investigated (1, 15, 30, 60 min), while *S. mutans* at successive stages of accumulation (12-24 h) was inhibited only by higher concentrations and longer exposure times. These data confirm the effective action of chitosan against *S. mutans* biofilms.

**25. Primary effusion lymphoma cells undergoing human herpesvirus type 8 productive infection produce C-type retroviral particles**

Pp 999-1006

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Primary effusion lymphomas (PELs) are invariably infected by the human herpesvirus 8 (HHV8) that is present in most PEL cells as latent virus but replicates in a subset of permissive cells to produce infectious progeny. Here we show that productively infected PEL cells release C-type retrovirus-like particles encoding an Mn<sup>++</sup>-dependent RT activity, which is typical of endogenous retroviruses. Strikingly, C-type particles are produced only in cells showing advanced HHV8 morphogenesis. Phorbol esters, which induce productive HHV8 replication and morphogenesis in PEL cells, increase RLP production. Phosphonoacetic acid, a blocker of HHV8 late gene expression, inhibits the production of C-type particles, whereas neutralizing anti- $\alpha$ IFN antibodies, which are known to increase HHV8 assembly, increases C-type particle production. These data suggest that factors expressed in advanced stages of HHV8 reactivation support endogenous C-type particle morphogenesis in PEL cells.

## 26. Can serum TGF-beta 1 be used to evaluate the response to antiviral therapy of haemophilic patients with HCV-related chronic hepatitis ?

Pp 1007-1012

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Congenital coagulation disorders limit the use of liver biopsy, especially when repeated assessment is needed. TGF-beta 1 plays a pivotal role in inducing fibrosis and has been proposed as its surrogate marker. Aiming at validating the clinical utility of this cytokine, fifteen haemophilic patients suffering from HCV-related chronic hepatitis were treated with Peg-IFN alpha2@plus Ribavirin. Serum TGFbeta 1, viral load and liver enzymes were analyzed at baseline and at six, twelve, and eighteen months. As expected, patients initially showed significantly higher TGF-beta 1 levels than age-matched controls (43.8 ng/mL, 28.7-46.4 vs. 26.9 ng/mL, 23.0-34.0, median and 95% CI; p=0.004). The end of therapy response rate was 67%. The main finding was a significant drop in TGF-beta 1 at six months compared to baseline values; this drop *de facto* predicted the levels reached in the following six months, which were fixed at lower concentrations (37.0 ng/mL, 21.9-43.8 and 27.0 ng/mL, 24.1-44.0 respectively; p<0.009), independently of treatment outcome (three patients were breakthrough, twelve were sustained virological responders (SVRs). During the treatment period none had clinical or biochemical signs of inflammation in other areas. Treatment was followed by a six-month follow-up, at the end of which TGF-beta 1 was increased compared to the previous values, reaching the initial levels in ten SVRs (45 ng/mL, 24.5-52.9). Interestingly, at a longer follow-up, two out of ten SVRs, who displayed the highest values of TGF-beta 1, relapsed. Serum TGF-beta 1 could be used to assess therapeutic outcome and short-term prognosis of HCV-related chronic hepatitis.

## 27. Antimicrobial property of a herbal preparation containing *Dalbergia sissoo* and *Datura tramonium* with cow urine against pathogenic bacteria

pp. 1013-1020

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In this study, a herbal preparation containing *Dalbergia sissoo* and *Datura stramonium* with cow urine (DSDS), was evaluated for its antibacterial potential against pathogenic strains of grampositive

(*Staphylococcus aureus* and *Streptococcus pneumoniae*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria. Antibacterial activity was compared to standard antibiotic drugs i.e. Chloramphenicol (30 mcg), Ampicillin (10 mcg), Nalidixic acid (10 mcg) and Rifampicin (30 mcg). Cow urine extract was found to be most active against both gram-positive as well as gram-negative bacteria. Clinical isolate of *S. aureus* showed higher sensitivity towards cow urine extract of DSDS than standard strains, and inhibited growth on most regulatory levels such as inhibition of protein, DNA, RNA and peptidoglycan synthesis. The results of the present study shows that the cow urine extract of DSDS may be used as a potent antiseptic preparation for prevention and treatment of chronic bacterial infections.

**28. Dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* has a protective effect against *Salmonella enteritidis* infection in mice**

Pp 1021-1029

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*Salmonella enteritidis* infection has received attention during recent years owing to its high prevalence worldwide. In the present study, the protective effect of probiotic dahi (curd) supplemented with *Lactobacillus acidophilus* and *L. casei* against *Salmonella enteritidis* infection in mice is investigated. Seven days pre-feeding with probiotic dahi significantly increased anti-*S. enteritidis* sIgA (secretory IgA) antibodies and lymphocyte proliferation in *S. enteritidis* infected mice. IL-2, IL-6 and IFN- $\gamma$  production were significantly increased in supernatant of cultured splenocytes collected from mice pre-fed with probiotic dahi, while IL-4 levels were not changed significantly. Moreover, activities of  $\beta$ -galactosidase and  $\beta$ -glucuronidase, and counts of *S. enteritidis* in intestine, liver and spleen were decreased, whereas total lactobacilli in faeces were increased in mice pre-fed with probiotic dahi. Pre-feeding of probiotic dahi for 7 days was more effective than 2 days pre-feeding. Thus, the results indicate that, pre-feeding with probiotic dahi ameliorated *S. enteritidis* infection by stimulating specific and non-specific immune response. Above all, it lowered colonization of gastrointestinal tract as well as translocation of *S. enteritidis*.

**29. *Enterobius vermicularis* infection of female genital tract**

Pp 1031-1033

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We report the case of female genital tract enterobiasis. The patient is a pediatrician at a large general hospital and was suffering from nocturia, dysuria and vaginal itching. Vaginal examination showed mild inflammation and normal hematogram. The midstream urine and the culture of vaginal discharge were negative. The Gram-stained microscopic examination of the vaginal discharge showed normal numbers of galactobacilli and absence of fungus, but the microscopic examination of fresh preparation of vaginal discharge revealed 3-4 leukocytes/mm<sup>3</sup> and *E. vermicularis* of about 4 mm. The patient was treated with

mebendazole 100 mg PO bid for 3 days and all the symptoms disappeared. In order to prevent possible re-infection, the treatment was repeated with a further two courses. After three months the cellotape test was negative.

**30. Low prevalence of selective iga deficiency in infected children born to HIV-seropositive mothers: an *in vivo* model for speculation on selective IgA deficiency pathogenesis**

Pp 1035-1039

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Anecdotal reports of restored immunoglobulin production in individuals with common variable immunodeficiency after acquiring HIV infection suggest that perturbation of the immune system occurring during HIV infection may force some underlying functional defects. These findings raise intriguing questions about the pathogenesis of common variable immunodeficiency. No study has investigated the possible influence of HIV infection on the development of selective IgA deficiency, a primary immunologic defect genetically related to common variable immunodeficiency. IgA serum levels were evaluated in a large cohort of children born to HIV-infected mothers from 1985 to 2006. To avoid differences possibly due to different follow-up durations we considered only infected and noninfected children aged over 4 years at last-follow-up. The study included 1,157 non-infected children and 964 infected children, aged  $\geq 4$  years at last-follow-up and with availability of two or more serum

**31. *Chlamydia Pneumoniae* and chronic diseases with a great impact on public health**

Pp 1041-1043

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*Chlamydia pneumoniae* is recognised as a common cause of respiratory tract infections and has recently been implicated in several extrapulmonary chronic diseases, with great impact on public health, such as atherosclerosis, multiple sclerosis and Alzheimer's disease. The involvement of *C. pneumoniae* in such diseases may be correlated to characteristic features of this pathogen, including intracellular growth and ability to induce persistent forms. *C. pneumoniae* persistent forms are inherently more suited to evade the host immune response and are more difficult to eradicate by antibiotics. Our preliminary experimental findings show that interaction of *C. pneumoniae* with macrophages and/or T cells characterized by interference with TNF- $\alpha$  production, and redox states, culminates in the induction of T cell apoptosis and survival of infected macrophages. Based on our evidence, the poor cooperation between T cells and macrophages could lead to an inappropriate immune response against *C. pneumoniae* that may therefore promote the development of extrapulmonary chronic diseases.

**32. Selective TNF-alpha gene silencing attenuates apoptosis in human salivary gland epithelial cells**  
Pp 1045-1047

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RNA interference (RNAi) was used in this study for selective knockdown of TNF-alpha gene expression in anti-Ro/SSA autoantibodies (Abs)-treated human salivary gland epithelial cells. Our findings reveal that selective TNF-alpha gene silencing resulted in the subsequent attenuation of the pro-apoptotic effects of anti-Ro/SSA Abs; this could have therapeutic effects in autoimmune diseases.

**33. Holly's story: illustration of an attention deficit hyperactivity disorder case**  
Pp 1049-1051

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During the last 10 years a significant increase of childhood neuropsychiatric disorders, such as attention deficit hyperactivity disorder (ADHD), has been reported. ADHD is believed to have a multifactorial etiology including multiple genetic and prenatal environmental factors. For this reason, there has been a recent revival regarding the role of autoimmunity in this pathology. An ADHD combined-type patient born from a drug-addicted mother was studied. Neuropsychological tests according to the criteria of the 4th edition of the Diagnostic and Statistical Manual (DSM4) permitted us to make the ADHD-diagnosis. The HLA-A, -B, and -DRB1 alleles of the child were determined by sequence-based typing (SBT) after DNA extraction. Although no autistic behavioral features were observed in the patient, a double genetic association between ADHD and autism was reported. In fact, HLA class I alleles (A\*02 and B\*44) associated to autism and the HLA class II allele (DRB1\*04) associated both to autism and ADHD were identified.