EDITORIAL

A CASE SERIES OF A LUTEOLIN FORMULATION (NEUROPROTEK®) IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

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There has been an impressive, little understood increase in cases of Autism Spectrum Disorders (ASD). The lack of any distinctive pathogenetic mechanism has hampered the development of any effective treatments. Increasing evidence indicates oxidative stress, brain inflammation, gastrointestinal (GI) dysfunction and allergic symptoms may be present in ASD patients. The flavone luteolin has anti-oxidant, anti-inflammatory, anti-allergy and neuroprotective properties. Given these findings, a dietary supplement was developed with a unique mixture of luteolin with the related flavonoids quercetin and rutin in a liposomal formulation of olive kernel oil (OKO), which increases their absorption. Results are presented for children with ASD (n=37, 4-14 years old) who had not obtained any benefit from multiple other regimens and who used this formulation for at least 4 months. GI and allergy symptoms improved in about 75% of children, eye contact and attention in 50%, social interaction in 25% and resumption of speech in about 10%. There were no adverse effects. Even though these results represent an uncontrolled open case series, they are encouraging because they suggest good tolerability and potential effectiveness.
Tendinopathies are very common in athletes and in people practicing sport activities. The experimental evidence that Growth Factors (GFs), present in platelets, enhance the recruitment, proliferation and differentiation of cells involved in tissue regeneration, has prompted the use of Platelet Rich Plasma (PRP) preparations in the treatment of these diseases. However, at present, a sound demonstration of the clinical efficacy of PRP is still lacking. Several theoretical and practical reasons can explain the failure of the treatment: a) animal experiments have been carried out on “normal tendons” submitted to surgical lesions, and it is questionable whether these models may best mimic human pathology; b) the pathway of chronic tendinopathies is very complex, involving, besides GFs, many other pathogenetic factors, which operate at different stages of the disease; c) several methods have been used to produce PRP, which can result in a large variation in GF content, and in kinetics of release. Therefore, further research is desirable. As a preliminary step, it is necessary to standardize PRP preparation, and to establish the modalities of its activation and administration. Secondly, prospective, randomized, double-blind studies are needed, selecting subjects with homogenous forms of tendinopathies: load-bearing and non-load-bearing tendons, midportion and insertional tendinopathies, with or without neovascularization. Finally, new strategies in PRP use should be exploited: among them, the association of PRP with autologous stem cells or the administration of selective GFs (fibroblast growth factor, vascular endothelial growth factor, or anti-angiogenic factors), which could be better options in specific situations.
EDITORIAL

INTERFERON-γ RELEASE ASSAYS FOR THE DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS INFECTION IN CHILDREN: A LITERATURE REVIEW

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The role of Interferon-gamma release assays (IGRAs) for immunologic diagnosis of tuberculosis in children is under debate. We carried out a narrative review on the studies on IGRAs in paediatric populations. A literature search was conducted using multiple keywords and standardized terminology in Medline, EMBASE and Cochrane databases, up to January 27th, 2011. Study quality was assessed using the MOOSE checklist and results of relevant studies were summarized. Sixty-seven paediatric studies (study population ranging from 14 to 5,244 children) were identified. Non-commercial ELISPOT assay (by means of ESAT-6 and CFP-10 antigens) had been carried out in 11 studies. QuantiFERON-TB Gold (QFT-G), QuantiFERON-TB Gold In-tube (QFT-G-IT), and T-SPOT.TB assays had been performed in 10, 44 and 18 studies, respectively. Most studies reported higher specificity of IGRA than tuberculin skin test (TST), but interpretation of the results is complicated by the fact that a gold standard for the diagnosis of latent TB is lacking. The reported sensitivity for active TB ranged from 51-93% for QFT-G/QFT-G-IT and 40-100% for ELISPOT assays, suggesting that a negative IGRA result may not exclude tuberculosis. Combining TST and IGRA results increased the diagnostic sensitivity. Rates of indeterminate results largely varied (0 to 35%). Most of the studies on young (< 5 years) or immune-compromised children reported a proportion of indeterminate results exceeding 4%. Agreement among TST and IGRA, assessed by the k statistics, ranged from -0.03 to 0.87. Higher rates of discordance were reported in BCG-vaccinated than in non-BCG-vaccinated children. Studies on children <5 years and immunocompromised children reported conflicting results, as did studies on serial IGRA determinations. Despite the large amount of literature data, the role of IGRA in the pediatric population is still unclear, especially in young children. Combined use of TST/IGRA may increase diagnostic sensitivity but interpretation of discordant results remains a challenging issue.
Alzheimer’s disease (AD) is a multifactorial disorder characterized by the progressive deterioration of neuronal networks. The primary cause and sequence of its progression are only partially understood but abnormalities in folding and accumulation of insoluble proteins such as β-amyloid and Tau-protein are both associated with the pathogenesis of AD. Mitochondria play a crucial role in cell survival and death, and changes in mitochondrial structure and/or function are related to many human diseases. Increasing evidence suggests that compromised mitochondrial function contributes to the aging process and thus may increase the risk of AD. Dysfunctional mitochondria contribute to reactive oxygen species which can lead to extensive macromolecule oxidative damage and the progression of amyloid pathology. Oxidative stress and amyloid toxicity leave neurons chemically vulnerable. The mitochondrial toxicity induced by β-amyloid is still not clear but may include numerous mechanisms, such as the increased permeability of mitochondrial membranes, the disruption of calcium homeostasis, the alteration of oxidative phosphorylation with a consequent overproduction of reactive oxygen species. Other mechanisms have been associated with the pathophysiology of AD. Inflammatory changes are observed in AD brain overall, particularly at the amyloid deposits, which are rich in activated microglia. Once stimulated, the microglia release a wide variety of pro-inflammatory mediators including cytokines, complement components and free radicals, all of which potentially contribute to further neuronal dysfunction and eventually death. Clinically, novel approaches to visualize early neuroinflammation in the human brain are needed to improve the monitoring and control of therapeutic strategies that target inflammatory and other pathological mechanisms. Similarly, there is growing interest in developing agents that modulate mitochondrial function.
It has been reported that high levels of cholesterol and triglycerides are associated with increased risk of developing atherosclerosis and shorter life. In fact, vascular endothelial dysfunction occurs during the human aging process. Accumulation of lipids in vascular endothelium activates leukocytes to produce cytokines and chemokines which recruit macrophages. On the other hand, macrophages augment inflammatory response and secrete vascular endothelial growth factor, a key cytokine that mediates angiogenesis and inflammatory response. In addition, hyperlipidaemia is one of the main risk factors for aging, hypertension and diabetes. Here, we review the interrelationship between endothelial cells, high level of cholesterol, and aging.
The aim of the present work is to investigate the link between two endocrine disruptor compounds (EDCs), which are chemicals that interfere with the hormone system in human and wildlife, and the human immune response through a study of their effects on the THP-1 human cell line which was used as a model for macrophages. We used two EDCs, diisononylphthalate (DiP) and 4-n-nonylphenol (NP) alone or in combination in order to evaluate the effects of these compounds on several parameters of the immune response - cytokine secretion, phagocytosis and the putative implication of the estrogen receptors - by studying the level of MAPK activation. NP and DiP strongly reduced phagocytosis and modify cytokine secretions. Indeed, differentiated THP-1 cell exposures (i) to 5 and 10 µM of combination of NP and DiP induced an IL-8 level in the medium respectively of 28.9 and 45% higher than the level obtained for the control (untreated cells), (ii) to combination of NP and DiP at 10 µM induced an increase of IL-1β level in comparison to the control level, (iii) to combination of NP and DiP induced an increase of TNF-α level whatever the concentration of EDCs tested (between 0 and 10 µM). Lastly, differentiated THP-1 cell exposure to NP, DiP alone or in combination (2 µM for each condition) induced a decrease of ERK1/2 phosphorylation in comparison to ERK1/2 phosphorylation level of the control. Moreover, differentiated THP-1 cell treatments by ICI-182780 (an estrogen receptor antagonist) supressed the EDC effects on ERK1/2 phosphorylation level which indicates an estrogen receptor-dependent pathway. These results suggest that EDCs have the ability to alter the human immune function, maybe by interfering with endocrine balance.
Matrix metalloproteinases (MMP) are a family of host-derived enzymes involved in the turnover of extracellular matrix (ECM) molecules, and, in particular, it is demonstrated that the 92 KDa gelatinase MMP-9 is often expressed in atherosclerotic plaques by macrophages and smooth muscle cells. Recent evidence supports a role of Toll-like receptor (TLR) signaling in the development of atherosclerosis lesions. In this study, we analyzed the TLR2/TLR4 expression in HUVEC infected with *C. pneumoniae* and correlated it to the production of VEGF and MMP-9. The results obtained showed an increased VEGF and MMP-9 production correlated with a time-dependent increase in cellular proliferation in HUVEC infected with *C. pneumoniae* at a multiplicity of infection (MOI) of 2 IFU/cell. HUVEC preincubated with VEGF antibody did not release MMP-9, as detected by zymography assessment and ELISA assay. In addition, we demonstrated that TLR2/TLR4 are expressed in HUVEC infected with viable microorganisms (25% and 17%, respectively), while UV-inactivated microorganisms induced a lesser expression (20% and 11%, respectively) compared to control cells and HUVEC exposed to heat-killed bacteria showed a percentage of TLR-expressing cells similar to the control cells. In addition, the cells preincubated for 60 min with TLR2/TLR4 neutralizing antibodies showed a decrease in *C. pneumoniae*-induced VEGF and MMP-9 production.
Hypertension has been suggested to exert pro-inflammatory actions through increased expression of several mediators, including chemokines. Chemokines are involved in inflammatory and autoimmune disorders, and in the formation of atherosclerotic lesions through promotion of inflammatory cell migration. The aim of this study is to evaluate the influence of high blood pressure on circulating levels of the prototype chemokines C-X-C motif ligand (CXCL)10 and C-C motif ligand (CCL)2 in 140 patients with essential hypertension not affected by thyroid disorders or overt autoimmune or inflammatory diseases, and 140 gender- and age-matched healthy controls. Mean CXCL10 and CCL2 levels were significantly higher in hypertensive patients than in controls. Among hypertensive patients, chemokines levels were higher in those with systo-diastolic hypertension compared to those with isolated systolic hypertension. In a multiple linear regression model using CXCL10 or CCL2 as dependent variables and age, body mass index, glycemia, serum creatinine, high-density-lipoprotein (HDL) and low-density-lipoprotein (LDL) cholesterol, triglycerides, and systolic or diastolic blood pressure values as covariates, only systolic or diastolic blood pressure values were significantly related to CXCL10 or CCL2 levels. In conclusion, this study demonstrates increased circulating levels of the prototype chemokines CXCL10 and CCL2 in patients with hypertension.
Orally administered immunomodulatory drugs have recently demonstrated the ability to induce an oral tolerance via inhibition of effector T cells and induction of certain subsets of regulatory T cells (Tregs) which have the potential to prevent several autoimmune diseases. In the present study, we hypothesized that short-term, low-dose, oral FTY720 administration may induce latency-associated peptide (LAP) Tregs and CD4^+ Foxp3^+ Tregs in atherogenesis, potentially resulting in remission of early development of atherosclerosis in apolipoprotein E-deficient (APOE^{-/-}) mice. FTY720 was orally administered to APOE^{-/-} mice 4 weeks of age on a high-cholesterol diet and atherosclerosis was assessed at 8 weeks of age. Oral administration of FTY720 significantly reduced atherosclerotic lesion formation compared with control mice. We observed a significant increase in LAP^+ and Foxp3^+ cells in the CD4^+ T-cell population of FTY720-treated mice in association with increased production of the anti-inflammatory cytokine transforming growth factor-β (TGF-β) as well as suppressed T-helper type 1 immune responses. Our findings reveal that short-term, low-dose oral FTY720 treatment had great benefits in inhibiting early development of atherosclerosis in mice via induction of a regulatory T-cell response and inhibition of effector T responses. These findings suggest that oral immune modulation may represent an attractive therapeutic approach to atherosclerosis.
Common variable immunodeficiency (CVID) is considered the most common symptomatic antibody deficiency and, although mainly reported in adults, it may present from childhood. Few data on the impact of TACI defects on the clinical and immunological status of children are available. We screened 42 hypogammaglobulinemic children to investigate the frequency and mutational features of TACI defects. The genetic, clinical and immunological characterization was extended to 31 relatives of 11 children with TACI mutations. Of interest, our analysis showed a considerably higher mutation frequency in hypogammaglobulinemic children (13/42; 31%) than in other cohorts of adult patients. In seven out of nine families with the C104R variant, the prevalence of autoimmunity was significantly higher in C104R heterozygous relatives (8/15; 53%) than in those with no C104R mutation (1/11; 9%). Our data suggest a different impact of TACI mutations, from hypogammaglobulinemia in children to autoimmune disease in adulthood.
Multicolor flow cytometry allows to study the markers differentially expressed during maturation, activation, function and senescence on immune cells. Despite the availability of reagents and technology, scarce agreement has been gained regarding phenotypic markers of HIV disease progression other than CD4 T-cell count. In this work, we present a novel high-throughput global analysis of CD4 and CD8 T-lymphocyte profiles by standardized 8-color combinations of antibodies aimed at analyzing HIV disease course progression. For this purpose, two tubes with lyophilized reagent cocktails (CD4- and CD8-specific tubes) were designed to compare the immunological characteristics of HIV-infected persons (37 “high CD4” HAART-treated and 32 “low CD4” naïve or failed-treatment patients) with healthy donors (HD). In particular, T-cell activation (CD25, CD38, CD69), differentiation (CD45RA, CCR7), apoptosis (CD95) and immune suppression profiles (CD25\textsuperscript{hi}CD127-) in HIV+ patients were compared with HD. Statistical analysis was performed by identifying the parameters associated with disease progression, namely markers that were found to be significantly different between groups with high CD4 counts (including HD) and low CD4 counts (restricted to HIV patients) but not between the HD and the “high CD4” group. This set of markers, including those identifying different maturation and senescence subtypes of CD4 and CD8 T cells, was found to be associated with therapy failure, and it is in fact evaluated in an ongoing study aimed to verify its prognostic value. This robust assay was found feasible on a semi-routine scale for HIV-infected persons, and allows for broader clinical studies aimed at defining markers associated with treatment outcome, possibly having a high impact on the clinical management of HIV disease.
The aim of this study is to evaluate some inflammatory parameter changes in septic shock patients and their possible correlation with clinical outcome, in particular when continuous veno-venous hemofiltration (CVVH) treatment is required. Considering the objective difficulty in enrolling this kind of patient, a preliminary study was initiated on seventeen septic shock patients admitted to a medical and surgical ICU. The mRNA expression of Toll-like receptor (TLR)-1, TLR-2, TLR-4, TLR-5, TLR-9, TNFα, IL-8 and IL-1β was assessed, the plasmatic concentrations of IL-18, IL-2, IL-10 and TNFα were measured on the day of sepsis diagnosis and after 72 h. In those patients who developed acute renal failure unresponsive to medical treatment and who underwent CVVH treatment the same parameters were measured every 24 h during CVVH and after completion of the treatment. On sepsis diagnosis, gene expression of TLRs was up-regulated compared to the housekeeping gene in all the patients. After 72 h, in 35% of the patients a down-regulation of these genes was found compared to day 1, but it was not associated with a reduction of cytokine serum levels or improved clinical signs, better outcome or reduced mortality. After high volume hemofiltration treatment, cytokine serum levels and TLR expression were not significantly modified. In conclusion, considering the not numerous number of cases, from our preliminary study, we cannot certainly correlate TLR over-expression in septic shock patients with severity or outcome scores.
A VALIDATED HPLC METHOD FOR THE MONITORING OF THIOPURINE METABOLITES IN WHOLE BLOOD IN PAEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Therapeutic drug monitoring (TDM) of major metabolites of thiopurine drugs is a widely used tool for assessing treatment efficacy and toxicity in patients with inflammatory bowel disease (IBD). We report the laboratory and clinical validation of a simple and reliable high performance liquid chromatography (HPLC) method for the measurement of 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MMP) on paediatric patients with IBD. The aim of this paper is to develop and validate a method for the measurement of 6-TGN and 6-MMP applicable to routine practice and to evaluate the usefulness of the TDM of thiopurine drugs in children with IBD attending our Gastroenterology Unit. The HPLC method was validated following international guidelines starting from red blood cells (RBC) and whole blood (WB). A comparison between RBC and WB was assessed. The usefulness of TDM was then evaluated using the new method from WB in 47 paediatric patients with IBD treated with thiopurine drugs. WB and RBC resulted in interchangeable matrices. The majority of patients had the metabolite levels inside the therapeutic ranges. A moderate correlation was found between 6-MMP concentration and the dose of thiopurines. A higher percentage of non responders was found among patients with lower levels of 6-TGN. Toxicity was found in eight patients and was evaluated in respect to the metabolite concentration. The described HPLC method is applicable to routine practice and it is suitable for its use in multicentric studies. Our results of TDM on paediatric IBD patients can contribute to clarify its role in their therapeutic management.
Natural rubber latex allergy (NRL-A) is an international problem of public health. About 50-60% of NRL-A patients may present adverse reactions after ingestion of cross-reacting vegetable foods. This condition, called “Latex-fruit Syndrome”, is a matter of research. The aim of our study is to distinguish between clinical/subclinical latex-fruit syndrome and cross-sensitization to latex and food/pollen allergens on the basis of latex recombinant allergens. We studied 51 patients with food hypersensitivity and serological evidence of NRL sensitization. The subjects underwent an accurate allergological evaluation (skin prick test with latex, food and pollen extracts, specific IgE to latex and recombinant allergens, challenge provocation tests). The patients were divided in two groups: group A) 34 patients with clinical and serological latex and fruit/vegetable allergies; group B) 17 patients allergic to fruits/vegetables and/or pollens, with serological, but not clinical NRL-A. All the latex challenge tests resulted positive in group A patients and only two patients of group B presented positive cutaneous challenge tests. Moreover, specific IgE-antibodies were detected to rHev b 5, to rHev b 6.01, to rHev b 6.02 and to rHev b 8 (and other profilins) of group A patients, while in group B we observed a monosensitization to Hev b8, probably linked to a cross-sensitization to pollens and foods. At the present state of knowledge, we need a multi-parametric approach based on a combination of clinical history, diagnostic tests (CRD) and latex challenge tests to make diagnosis of latex-fruit syndrome.
Rheumatoid arthritis (RA) is a debilitating autoimmune disease of global prevalence and the disease process primarily targets the synovial joints. Despite improvements in the treatment of RA over the past decade, there still is a need for new therapeutic agents that are efficacious, less expensive, and free of severe adverse reactions. Celastrus has been used in China for centuries for the treatment of rheumatic diseases. Furthermore, we previously reported that ethanol extract of *Celastrus aculeatus* Merr. (Celastrus) attenuates adjuvant-induced arthritis (AA) in rats. However, the mechanisms underlying the anti-arthritic activity of Celastrus have not yet been fully defined. We reasoned that microarray analysis might offer useful insights into the pathways and molecules targeted by Celastrus. We compared the gene expression profiles of the draining lymph node cells (LNC) of Celastrus-treated (Tc) versus water-treated (Tw) rats, and each group with untreated arthritic rats (T₀). LNC were restimulated with mycobacterial heat shock protein-65 (Bhsp65). We identified 104 differentially expressed genes (DEG) (8 upregulated, 96 downregulated) when comparing Tc with T₀ rats, in contrast to 28 (12 upregulated, 16 downregulated) when comparing Tw and T₀ rats. Further, 20 genes (6 upregulated, 14 downregulated) were shared by both Tw and Tc groups. Thus, Celastrus treatment (Tc) significantly downregulated a large proportion of genes compared to controls (Tw). The DEG were mainly associated with the processes of immune response, cell proliferation and apoptosis, and cell signaling. These results provide novel insights into the mechanism of Celastrus anti-arthritic activity, and unravel potential therapeutic targets for arthritis.
MICRORNA 143-145 DEFICIENCY IMPAIRS VASCULAR FUNCTION

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MicroRNAs are required for vascular smooth muscle growth, differentiation and function. MiR143-145 modulates cytoskeletal dynamics and acquisition of the contractile phenotype by smooth muscle cells. Lack of this miRNA cluster results in decreased blood pressure and reduced vasocontraction. As all these observations point to a key role for miR143-145 in the vasculature, we investigated whether miR143-145 deficiency is associated with impaired vascular tone. Vasocontraction was assessed in isolated aortic rings from miR143-145 KO and wild type animals incubated with increasing concentrations of phenylephrine (10⁻⁹ M to 10⁻⁵ M) or KCl 0.3 M. In both cases, aortic vessel contraction was dramatically reduced in miR143-145 KO animals compared to controls. Next, aortic rings were pre-contracted with phenylephrine (EC60: 10⁻⁷ M) and concentration responses for acetylcholine were obtained. A significantly reduced vasodilation was observed in miR143-145 KO animals compared to controls and similar results were obtained when an exogenous donor of nitric oxide (sodium nitroprusside) was used. Endothelial nitric oxide synthase or guanylate cyclase mRNA expression were not different between the animal groups thus suggesting to investigate the effect of other vasodilators. Isoprenaline mediated vasodilation was significantly reduced in miR143-145 KO animals compared to controls and in the absence or in the presence of the guanylate cyclase inhibitor ODQ (10⁻⁴ M), suggesting that also beta adrenergic vasodilation is impaired following miR143-145 deficiency. Finally, the effect of a stable mimetic prostacyclin, namely iloprost, was investigated and again a reduced vasodilation was observed in miR143-145 KO animals. MiR143-145 deficiency is associated not only with altered vasocontraction but also with impaired vasodilation, which probably reflects the impaired VSMC differentiation phenotype reported in miR143-145 KO animals.
OMALIZUMAB MODULATES BRONCHIAL RETICULAR BASEMENT MEMBRANE THICKNESS AND EOSINOPHIL INFILTRATION IN SEVERE PERSISTENT ALLERGIC ASTHMA PATIENTS

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Severe persistent asthma causes a substantial morbidity and mortality burden and is frequently not well controlled, despite intensive guideline-based therapy. The unique monoclonal antibody approved for patients with severe allergic asthma is omalizumab: a recombinant humanised murine against IgE antibodies. The aim of the present study is to investigate the effect of long-term anti-IgE on the thickening of the reticular basement membrane (RBM) and eosinophil infiltration in bronchial biopsies from patients with severe persistent allergic asthma. Biopsies were obtained from 11 patients with severe persistent allergic asthma before and after (12 months) treatment with omalizumab. RBM thickness and eosinophils were measured by using light microscope image analysis. A significant mean reduction in RBM thickness and eosinophil infiltration were measured after one-year omalizumab treatment. No correlation between eosinophil reduction and RBM thickness reduction was found. No correlation between each of the previous two parameters and clinical parameters was detected. In conclusion, our study showed that a substantial proportion of severe asthmatics reduced the original bronchial RBM thickness and eosinophil infiltration after one-year treatment with anti-IgE, thus emphasizing the possible role of omalizumab in affecting airway remodeling in severe persistent allergic asthma.
LETTER TO THE EDITOR

EFFECTIVENESS OF NEBULIZED HYPERTONIC SALINE AND EPINEPHRINE IN HOSPITALIZED INFANTS WITH BRONCHIOLITIS

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The objective of the study is to verify effects of nebulized 3% saline hypertonic solution (HS) in comparison to normal saline (NS) in addition to epinephrine in hospitalized children with bronchiolitis. Infants were randomly assigned either to receive every 6 hours nebulized NS (group I) or 3% HS (group II) in addition to epinephrine (1.5 mg) and to conventional treatment. The main endpoints of this study were the length of stay (LOS) in hospital and the clinical response score (CSS). Patients presented a significant decrease in CSS from the first through the third day of treatment, present in the first group but even more evident in the second group (p=0.0001). Comparison between group I and II data shows significant decrease in CSS in the 3% HS-treated patients both at the second (p<0.005) and at the third day of treatment (p<0.005). Infants in the NS control group had a mean LOS of 5.6±1.6 days, whereas children treated with 3% HS were discharged with a LOS of 4.9±1.3 days, reaching a significant decrease in stay (p<0.05). In hospitalized patients bronchiolitis nebulized 3% HS and epinephrine significantly decreased symptoms and LOS as compared to 0.9% NS and epinephrine.
Bronchiolitis is a lower respiratory tract viral infection which may result in severe bronchial obstruction and respiratory failure despite treatment with beta-adrenergic agonists and glucocorticoids. Here we describe two otherwise healthy infants with severe bronchiolitis whose clinical course was complicated by marked bronchial obstruction and respiratory acidosis refractory to conventional medications (β-stimulants, anticholinergics and corticosteroids) and non-invasive positive pressure ventilation. Sevoflurane inhalation allowed both infants to attain a sustained, clinical improvement in ventilation and one patient to avoid mechanical ventilation. We suggest that sevoflurane inhalation may be a therapeutic option in the treatment of young infants with severe bronchiolitis who respond poorly to conventional therapy.
LETTER TO THE EDITOR

PSORIASIFORM DERMATITIS IN A NON-PSORIATIC PATIENT TREATED WITH ADALIMUMAB

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Anti-TNF drugs may be associated with various adverse reactions including cutaneous ones. We describe the case of a 45-year-old woman affected by undifferentiated spondyloarthritis who presented a localized psoriasiformis dermatitis during treatment with adalimumab, without any medical history of psoriasis.
Orofacial granulomatosis (OFG) is a clinicopathologic entity describing oral lesions with noncaseating granulomas including a spectrum of diseases such as the Melkersson-Rosenthal syndrome. The involvement of abnormal T-cell responses has been suggested in the pathogenesis of OFG although few and contrasting data are currently available on this issue. In a patient with OFG, we observed virtually complete CD4 and CD8 T-cell receptor (TCR) β-chain variable region (BV) repertoires at the lesion level and in circulation. However, oligoclonal profiles were found in CD4 and, to a greater extent, in CD8 subsets. These findings were seen in association with a massive peripheral T-cell activation, decreased naive T cells, reduced thymic output, altered cytokine production, and increased apoptosis. Our data, pointing to a random influx of T cells at the site of inflammation, argue against the hypothesis of a main allergen acting at the level of oral mucosa. The profound dysregulation of the peripheral T-cell compartment suggests that OFG should be regarded as a systemic disorder with localized manifestations.
LETTER TO THE EDITOR

ACQUIRED PERIPHERAL NEUROPATHY: A REPORT ON 20 CHILDREN

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Guillain–Barré syndrome (GBS) is an inflammatory polyneuropathy characterized by acute onset, rapid progression, symmetric muscular weakness, pain, and paresthesias. The incidence of GBS in the pediatric age group is 0.8 cases per 100,000; 50%-70% of the cases are preceded by respiratory or gastrointestinal infectious episodes or vaccination. The etiopathogenesis of GBS has been hypothesized to involve a direct immune-mediated mechanism against the peripheral nerves. A series of 20 patients managed in the Department of Pediatrics of the University of Catania between 2003 and 2011 and evaluated according to epidemiologic, clinical, and therapeutic features is reported.
LETTER TO THE EDITOR

EFFECTS OF ORAL HYPOSENSITIZATION THERAPY WITH NICKEL ON HAEMOGLOBIN AND HAEMATOCRIT VALUES IN SENSITIZED PATIENTS

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Nickel deficiency leads to reduced iron content in organs and to reduced hemoglobin levels and haematocrit counts. We carried out a clinical trial of oral hyposensitization therapy with low doses of nickel on a group of 20 patients affected by systemic allergy to this sensitizer element and with a chronic relapsing low hemoglobin level and haematocrit count. We obtained interesting results on maintaining these values in treated patients.
LETTER TO THE EDITOR

ABSENCE OF XENOTROPIC MURINE LEUKEMIA VIRUS-RELATED VIRUS IN ITALIAN PATIENTS AFFECTED BY CHRONIC FATIGUE SYNDROME, FIBROMYALGIA, OR RHEUMATOID ARTHRITIS

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The xenotropic murine leukemia virus-related virus (XMRV) has been recently linked to chronic fatigue syndrome in a US cohort in whom the virus was demonstrated in 67% patients vs 3.7% healthy controls. Albeit this finding was not substantiated by subsequent reports and eventually considered a laboratory contamination, the matter is still the object of intense debate and scrutiny in various cohorts of patients. In this work we examined well-clinically characterized Italian patients affected by chronic fatigue syndrome, and also fibromyalgia and rheumatoid arthritis, two chronic illnesses of basically unknown etiology which show quite a few symptoms in common with chronic fatigue syndrome. Although we used recently updated procedures and controls, the XMRV was not found in 65 patients with chronic fatigue syndrome diagnosis, 55 with fibromyalgia, 25 with rheumatoid arthritis, nor in 25 healthy controls. These results add to the ever-growing number of surveys reporting the absence of XMRV in chronic fatigue syndrome patients and suggest that the virus is also absent in fibromyalgia and rheumatoid arthritis.
Profilins are “panallergens”, responsible for many cross-reactivities between inhalant, latex and plant-derived food allergens. We evaluated the effectiveness and the safety of sublingual desensitization treatment (SLIT) in two patients with allergic respiratory and food diseases. Skin prick tests, IgE and IgG4 assays to pollens, some plant-derived foods, profilin, non-lipid specific transfer protein and PR10 proteins were performed. The patients also underwent double-blind placebo-controlled challenge (DBPCFC) with the culprit foods and profilin and then a SLIT with it. Both the patients had positive SPT, specific IgE and DBPCFCs with profilin and some vegetables referred in anamnesis. They therefore underwent SLIT with profilin extract. At the end of treatment, the patients had negative DBPCFCs with culprit foods and a decrease of specific IgE levels for profilin and vegetable foods. Profilin desensitization allowed our patients to manage their diet without restriction, eating several foods previously not tolerated.
LETTER TO THE EDITOR

LASER REMOVAL OF TATTOOS

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In Western countries the phenomenon of “tattooing” is expanding and tattoos are considered a new fashion among young people. In this paper we briefly trace the history of tattooing, the techniques used, the analysis of pigments used, and their possible adverse reactions. We also carried out a review of the international literature on the use of Q-switched laser in tattoo removal and its complications, and we describe our experience in the use of this technique.
LETTER TO THE EDITOR

SALBUTAMOL: HOW DOES IT ENTER SMOOTH MUSCLE CELLS?

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Polyspecific organic cation transporters (OCTs) in human cell membranes are involved in the uptake, distribution and excretion of cationic compounds. Although their relevance to drug disposition in the liver, small intestine and kidney has been investigated previously, less is known about the influence of these transporters on the pharmacokinetics and pharmacodynamics of inhaled drugs. Drugs that are commonly administered by inhalation for the treatment of respiratory diseases, such as glucocorticoids and cationic β2-agonists, might interact with several of these transporters, which are strongly expressed on the surfaces of airway epithelial cells. We evaluated the expression of OCT3 and measured the in vitro uptake of the short-acting β2-agonist salbutamol (SALB), alone or in combination with corticosterone (CS) and beclomethasone dipropionate (BDP), by bronchial smooth muscle cells. Our results showed that these cells express the OCT3 transporter and that SALB enters the cell in a transporter-independent fashion. Moreover, CS and BDP have different activities on SALB transport inside the cell. CS increases SALB transport and BDP decreases SALB transport, although neither of these effects are statistically significant. A better understanding of these mechanisms might lead to the improved treatment of airway diseases.
LETTER TO THE EDITOR

TYPE 1 DIABETES ONSET AND PANDEMIC INFLUENZA A (H1N1)

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Type 1 diabetes (T1D) is a heterogeneous disorder characterized by destruction of pancreatic beta cells, culminating in loss of insulin secretion. Data from large epidemiologic studies worldwide indicate that during the last decades the incidence of T1D has increased significantly, reaching percentages of 2–5% annually. This increase suggests that there is a significant environmental contribution impacting the development of the disease, since genetic factors alone can hardly explain the rapid increase. Studies regarding T1D epidemiology in diverse populations aim to identify the disease causal factors and new targets for intervention. Viruses are one of the environmental factors implicated in the development of T1D in susceptible individuals. Recent studies suggest an association of T1D with H1N1 influenza. We would like to comment on this association and report our experience. Prospective studies are necessary to assess whether H1N1 infection is involved in T1D pathogenesis and provide directions on how to deal with viral infections in diabetes-susceptible individuals.
LETTER TO THE EDITOR

DISTORTION-PRODUCT OTOACOUSTIC EMISSIONS: A USEFUL TEST FOR MONITORING OTOTOXICITY INDUCED BY PEGYLATED INTERFERON AND RIBAVIRIN TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C

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Pegylated-interferon (peg-IFN) and ribavirin combination therapy for the treatment of hepatitis C virus (HCV) infection is well known to be associated with significant adverse effects. Several studies have investigated a possible auditory pathway involvement during IFN therapy, but a method to monitor the potential auditory involvement during treatment has not yet been described. The aim of this study is to evaluate possible modifications of the outer hair cell (OHC) function in HCV patients receiving peg-IFN and ribavirin combination therapy. Thirteen adult HCV patients (8 F/5 M, mean age 52±12 years) treated with peg-IFN and ribavirin combination therapy underwent Pure Tone Audiogram and Distortion Product Otoacoustic Emission (DPOAE) tests. We compared mean auditory thresholds (PTA) and mean DPOAE amplitude before, at month 3 during, and at the end of treatment (T0, T3, and Tend, respectively), and 3 months after treatment discontinuation (Tfu). No significant differences were found in hearing levels at the different time points analyzed. During treatment, three patients developed tinnitus, which in 2 cases resolved spontaneously after the end of therapy. Compared to T0 (19.5±0.83), a statistically significant DPOAE increase at T3 (30±1.26) and Tend (28.6±2.16) was found (p<0.05 at both time points), while DPOAEs returned to pre-treatment levels at Tfu (19.3±1.3). In our group, none of the patients reported a permanent auditory impairment, excluding one patient with persistent tinnitus. Peg-IFN could produce an increase of motility of the OHCs by means of intracellular pathways. DPOAE test could be considered a new method for monitoring ototoxicity induced by IFN. On the basis of recent literature and our audiological results, physicians should be aware of the possible ototoxic effects of peg-IFN, requiring appropriate surveillance, and the patient should be informed of the potential side effects of IFN therapy on the auditory pathway.