ANALYSIS ON THE CO-LOCALIZATION OF ASBESTOS BODIES AND FAS OR CD163 EXPRESSION IN ASBESTOS LUNG TISSUE BY IN-AIR MICRO-PIXE

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To prevent and control diseases caused by exposure to various agents, it is necessary to determine the harmful level for intervention and to establish a method for measuring that level. In-air microparticle-induced X-ray emission (in-air micro-PIXE) analysis is based on irradiation of specimens with a proton ion microbeam, and has been modified for biological application. Two-dimensional analysis and quantitative analysis using the system confirmed that asbestos induced apoptosis by upregulating Fas expression and also revealed the accumulation of CD163-expressing macrophages in the lungs of patients with asbestosis. By quantitative comparison of the area of Fas or CD163 expression and the Fas- or CD163-negative area in asbestos lung tissue, the harmful levels which caused the expression of Fas or CD163 could be estimated on Silica, Ferrous iron, and Magnesium (the components of asbestos) deposition. These results indicate that the system could be useful for investigating the pathogenesis of inhaled particle-induced immune reactions and for determining harmful levels of exogenous agents.

TUNEL AND GROWTH FACTOR EXPRESSION IN THE PREFRONTAL CORTEX OF ALZHEIMER PATIENTS OVER 80 YEARS OLD

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To elucidate factors underlying the increased risk of developing Alzheimer’s disease (AD) in older individuals, the prefrontal cortices of younger (58-79 years) and of older (over 80 years) AD patients were examined by silver impregnation, TUNEL assay and immunohistochemistry for hyperphosphorylated tau, LDH and two growth factors (BDNF, NGF). Quantitative data were compared with those of age-matched controls. TUNEL-positive cells were mainly located in superficial cortical layers of younger and in deeper layers of older AD patients. Their density was more than 5 times higher in older AD than in younger AD (p ≤ .05), but apoptotic cell morphology was rarely seen. Significantly more neuronal somas were contacted by degenerating fibers both in younger and older AD cortices. Density of tau-immunoreactive cells, which were virtually absent in controls, was twice as high in older AD patients as in younger AD individuals (p ≤ .05). In younger AD, TUNEL positive cells generally lacked tau immunoreaction, whereas in older AD, most cells were double-labeled for hyperphosphorylated tau and TUNEL (p ≤ .05). Numerical density of BDNF-immunoreactive cells was significantly reduced by 20% in older AD patients, compared to both control individuals and younger AD patients, whereas density of NGF-positive cells was the same in all patient groups examined. The distinct differences between younger and older AD patients suggest a faster progression of AD in older patients.
DETAILED CHARACTERISATION OF CB2 RECEPTOR PROTEIN EXPRESSION IN PERIPHERAL BLOOD IMMUNE CELLS FROM HEALTHY HUMAN VOLUNTEERS USING FLOW CYTOMETRY

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It is commonly accepted from gene expression studies that the CB2 receptor is expressed by most cell types of the rodent and human immune system. However, the exact identity of cells expressing CB2 receptor protein in human blood or the abundance of receptors expressed by each immune subset is not well characterised. We conducted a detailed analysis of CB2 protein levels expressed by blood-derived immune cells from healthy human donors. Flow-cytometry was conducted using 4 commercially available anti-CB2 polyclonal antibodies in conjunction with a selection of immune cell specific markers. Across multiple healthy subjects we observed that NK cells, B-lymphocytes and monocytes expressed a higher level of CB2 receptor than CD4⁺ or CD8⁺ T-lymphocytes. Neutrophils also expressed a low level of CB2 receptor. NK cells had the greatest variation in CB2 expression levels, whereas for each of the other cell types CB2 levels were relatively similar between subjects. In contrast to other methods, the high sensitivity of flow-cytometry revealed that CB2 receptors are present on resting T-lymphocytes at low abundance in some healthy subjects. These data provide the first detailed analysis of CB2 protein levels in blood leukocyte subsets from healthy donors and identifies the cell types which could be targeted with CB2-mimetic drugs in humans.

INCREASED TUMOR-SPECIFIC CD8⁺ T CELL INDUCTION BY DENDRITIC CELLS MATURED WITH A CLINICAL GRADE TLR-AGONIST IN COMBINATION WITH IFN-γ

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The limited response rate of cancer patients treated with dendritic cell (DC)-based vaccines indicates that vast improvements remain necessary. In many murine tumour models it has been demonstrated that the use of innate triggers (e.g. TLR triggers) in the maturation of DC results in higher efficacy. However, as few of these innate triggers are generated clinical grade, there remains a great necessity to fill the gap between fundamental mouse studies and a clinical trial in humans. In the present study we used a TLR2/4-agonist (FMKp which is available clinical grade) in combination with IFN-γ (FI-cocktail) in the maturation of elutriated monocyte-derived DC and compared it with the most used DC in current clinical trials (TNF-α/PGE-2, i.e. TP-cocktail). In addition to the assessment of CD4⁺ T cell polarizing capacity, we compared the quantity and intrinsic quality of induced CD8⁺ T cells of 2 different DC maturation protocols with all cells from the same donor. Besides differences in the cytokine profile, which could be coupled to increased Th1 and Th17 polarization, we demonstrate in this study that FMKp/IFN-γ matured DC are twice as effective in inducing cytotoxic T cells against known tumor antigens. Both DCs induced phenotypically equivalent effector memory CD8⁺ T cells that did not show a significant difference in their intrinsic capacity to kill tumor cells. These findings point to the therapeutic applicability of FI-DC as superior inducers of functional antigen-specific T cells. Their increased chemokine secretion is suggestive of a mechanism by which these DC may compensate for the limited migration observed for all ex vivo cultured DC when applied in patients.
 Periodontitis is a chronic infectious disease, characterized by the progressive loss of the teeth’s supporting tissues, affecting almost 40% of the entire adult population. An imbalance between a localized gram-negative infection and an exaggerated host inflammatory response plays a pivotal role in determining gingival tissue damage. Recent evidence suggests that the effect of periodontitis might not be limited just to the oral cavity but it might have systemic consequences. Indeed periodontitis has also been associated with a moderate systemic inflammatory response. Although the mechanisms behind this association remain unclear, periodontitis might represent one distant source of low-grade systemic inflammation. This association could explain the increased risk of future cardiovascular diseases observed, the impaired metabolic control in diabetes subjects and the adverse pregnancy outcomes observed in populations suffering from periodontitis. In this review we describe the pathophysiological processes involved in periodontitis and briefly review the evidence produced to support an association between periodontitis and systemic diseases.

PERIODONTITIS: FROM LOCAL INFECTION TO SYSTEMIC DISEASES

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Periodontitis is a chronic infectious disease, characterized by the progressive loss of the teeth’s supporting tissues, affecting almost 40% of the entire adult population. An imbalance between a localized gram-negative infection and an exaggerated host inflammatory response plays a pivotal role in determining gingival tissue damage. Recent evidence suggests that the effect of periodontitis might not be limited just to the oral cavity but it might have systemic consequences. Indeed periodontitis has also been associated with a moderate systemic inflammatory response. Although the mechanisms behind this association remain unclear, periodontitis might represent one distant source of low-grade systemic inflammation. This association could explain the increased risk of future cardiovascular diseases observed, the impaired metabolic control in diabetes subjects and the adverse pregnancy outcomes observed in populations suffering from periodontitis. In this review we describe the pathophysiological processes involved in periodontitis and briefly review the evidence produced to support an association between periodontitis and systemic diseases.

BETA2-GLYCOPROTEIN I IS A TARGET OF T CELL REACTIVITY IN PATIENTS WITH ADVANCED CAROTID ATHEROSCLEROTIC PLAQUES

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Evidence in animal models that beta2-glycoprotein I (β2GPI), the principal target of autoimmune antiphospholipid antibodies, is involved in the initiation and progression of atherosclerosis, prompted us to investigate the possible role of this self protein as a target autoantigen of immune reactions in patients with carotid atherosclerosis. Plaque-infiltrating T lymphocytes from patients, and circulating T lymphocytes from patients and healthy subjects were tested by cell proliferation assay and by flow cytometry for intracellular cytokine expression in response to β2GPI. ELISA was used to detect cytokine production in culture supernatants and anti-β2GPI/anti-cardiolipin antibodies in serum samples. Eight of 35 PBMC samples and 1 of 5 plaque-infiltrating T lymphocyte samples from patients proliferated in response to β2GPI, whereas PBMC from healthy subjects did not. Patients’ PBMC samples that proliferated in response to β2GPI produced significantly higher IFN-γ and TNF-α than non-proliferating PBMC. β2GPI-specific plaque-derived T lymphocytes expressed IFN-γ, TNF-α and IL-4, suggesting concomitant Th1 and Th2 activation. Only one patient’s serum was positive for anti-β2GPI and anti-cardiolipin IgM antibodies. These new findings indicate that β2GPI induces a cellular immune response in a subpopulation of patients with carotid atherosclerosis thus contributing to the inflammatory responses involved in carotid atherosclerotic disease.
ESSENTIAL AMINO ACIDS IMPROVE INSULIN ACTIVATION OF AKT/MTOR SIGNALING IN SOLEUS MUSCLE OF AGED RATS

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Essential amino acids (EAA) improve basal muscle protein synthesis in the elderly. Nevertheless, in settings of prolonged supplementation, putative signal pathways of EAA are currently unknown. The purpose of this study was to test the effects of prolonged supplementation of EAA enriched mixture (12-L-Amin) on Insulin/Insulin-like Growth Factor-1 (IGF1) pathway by measuring total and phosphorylated Akt(Ser⁴⁷³) and its upstream (IRS1 at Ser⁶³⁶) and downstream (mTOR at Ser²⁴⁴⁸, p70S6K at Thr³⁸⁹) targets in basal conditions and following acute insulin (0.1 U/L) incubation in vitro. To this aim, soleus muscles were dissected from male Wistar rats divided in three groups of 7 each: adults (AD, 10 mo of age), elderly (EL, 22 mo of age) and elderly supplemented (EL-AA, 12-L-Amin 1.5gr/Kg die in drinking water for 3mo). EL showed reduced basal and post-insulin mTOR and p70S6K activation and reduced post-insulin IRS1 degradation relative to AD. EL-AA showed an increase of post-insulin Akt activation, no change in basal and post-insulin phospho-mTOR, lower reduction of phospho-p70S6K and increased post-insulin IRS1 degradation relative to AD. These results demonstrate that chronic 12-L-Amin administration exerts anti-ageing effects on the activation/inactivation of the Insulin/IGF1/mTOR pathway which is identified as putative target of EAA in the elderly.

ERYTHROPOIESIS EQUIVALENCE, PHARMACOKINETICS AND IMMUNE RESPONSE FOLLOWING REPEAT HEMATIDE™ ADMINISTRATION IN CYNOMOLGUS MONKEYS

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Hematide™ is a synthetic PEGylated peptidic erythropoiesis stimulating agent (ESA) that is presently being developed for the correction of anemia in patients with chronic renal failure. Unlike currently marketed ESAs, Hematide does not possess any sequence homology to erythropoietin (EPO) and has not elicited moribund immune responses in animal safety studies thereby allowing the generation of a robust safety package. Animals administered marketed ESAs develop anti-EPO antibodies that null the effect of the administered ESA and neutralize endogenous EPO, resulting in severe anemia that precludes the interpretation of chronic safety studies. The primary objective of this study is to determine whether Hematide-specific antibodies are generated when male monkeys are exposed to high Hematide doses (10 mg/kg, intravenous [IV] and subcutaneous [SC]) administered at frequent dosing intervals (every two weeks) for a total of 9 doses; secondary objectives are to evaluate whether developed antibodies impact pharmacokinetics (PK) and pharmacology. In this study, no Hematide-specific antibodies were detected. Hematide exhibits a prolonged plasma half-life and slow clearance by either IV or SC administration. Hematide induced significant erythropoiesis with reticulocytosis and subsequent increases in red blood cells, hematocrit and hemoglobin (Hgb) levels. No erythropoietic differences were noted between the IV and the SC dosed groups with mean ± SD Hgb levels of 20.9 ± 2.5 and 20.3 ± 2.1 g/dL, respectively, occurring on Day 48, corresponding to Hgb increases of 6.5 and 6.7 g/dL, respectively, over pre-dose levels. In conclusion, Hematide is a potent erythropoiesis stimulating agent that exhibits plasma persistence in monkeys. Similar erythropoietic responses were produced following IV and SC administration. The absence of antibody development suggests that Hematide, at the doses and regimen described, has a low immunogenic potential in cynomolgus monkeys.
Nano-sized particles are diffusing in the environment with the development of nanotechnology. Polystyrene (PS) nanoparticles are modified industrial products and pharmaceutical agents, however, adverse effects of PS nanoparticles remain to be elucidated. In the present study, we investigated the effects of PS nanoparticles with different sizes on the atopic dermatitis (AD)-like skin lesions in NC/Nga mice assumed to show the skin barrier defect/dysfunction in the presence or absence of mite allergen. Male NC/Nga mice were injected intradermally with three different-sized PS nanoparticles (25, 50, or 100 nm) and/or mite allergen into their right ears. We evaluated clinical scores, ear thickening, histological findings and the local protein expression of inflammatory molecules in the ear and Ig production in serum. PS nanoparticles aggravated AD-like skin lesions related to mite allergen, which was paralleled by the local protein levels of interleukin-4, CCL2/monocyte chemotactic protein-1, CCL3/macrophage inflammatory protein-1 alpha, and CCL4/macrophage inflammatory protein-1 beta. In contrast, PS nanoparticles decreased interferon-γ expression. Furthermore, exposure to PS nanoparticles induced ear swelling and CC-chemokine expression in the absence of allergen. These effects were greater with the smaller PS nanoparticles than with the larger ones regarding overall trend. These results suggest that exposure to PS nanoparticles under skin barrier defect/dysfunction can exacerbate AD-like skin lesions related to mite allergen in a size-dependent manner. The enhancing effects may be accounted for by T helper 2-biased immune responses. Furthermore, PS nanoparticles can evoke skin inflammation via the overexpression of CC-chemokines even in the absence of allergen in atopic subjects.
Efficacy of a Spray Compound Containing a Pool of Collagen Precursor Synthetic Aminoacids (L-Proline, L-Leucine, L-Lysine and Glycine) Combined with Sodium Hyaluronate to Manage Chemotherapy/Radiotherapy-Induced Oral Mucositis: Preliminary Data of an Open Clinical Trial

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Oral mucositis (OM) is a very frequent and potentially severe complication experienced by patients receiving chemotherapy and/or radiotherapy, which often leads to significant morbidity and mortality, and decreased quality of life, and is very costly. Despite its severity and prevalence, there is no standard recognised management today. The aim of this open clinical trial is to evaluate the efficacy and compliance of a new spray compound containing sodium hyaluronate (SH) and a pool of collagen precursor amino acids (AAs) combined with sodium hyaluronate (SH) to manage radio/chemotherapy-induced OM. Twenty-seven consecutive patients with OM were treated according to the manufacturer’s instructions. At time T0 (baseline – before intervention), we evaluated the following parameters: (i) pain score (by linear visual analogue scale; 0–100) and (ii) severity of OM scored according to WHO Mucositis scale. The treatment efficacy was evaluated on i) pain score, ii) clinical resolution index (CRI) and iii) patient compliance at times T01 (after 2 hours), T1 (after 24 hours), T2 (after 72 hours), T3 (after 7 days) and T4 (after 14 days). Results showed that painful symptoms were significantly reduced after only 2 hours of spray administration compared with baseline measurements ($p<0.0001; z=-4.541$). A progressive reduction of pain through the 2 weeks was also noted ($p<0.0001$). Patient lesions treated with SH-AAs-based spray also significantly improved after 72 hours of treatment ($p=0.0051; z=-2.803$). During the two-week observation, all patients significantly improved from the baseline ($p<0.0001$) and progressively ameliorated their ability to swallow foods and liquids. The compliance of all patients to the product was very good, and at the end of the study there were no adverse effects. The results suggest that the SH-AAs-based spray accelerates lesion healing and above all helps to manage mucositis pain, especially in terms of immediate pain relief (after 2 hours from application). Although further randomized controlled studies are recommended, our findings suggest that frequent applications of this spray may offer rapid and effective pain management, aiding faster mucosal wound healing.
CLINICOPATHOLOGICAL AND THERAPEUTIC SIGNIFICANCE OF CXCL12 EXPRESSION IN LUNG CANCER

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Interactions between CXCL12 and its receptors CXCR4 or CXCR7 are involved in tumor growth and metastasis in various types of human cancer. However, CXCL12 expression and its role in lung cancer are not fully elucidated. Here we examined the expression of CXCL12 in 54 lung cancer cell lines consisting of 23 small cell lung cancers (SCLCs) and 31 non-small cell lung cancers (NSCLCs). CXCL12 was overexpressed in lung cancer cell lines compared to non-malignant human bronchial epithelial cell lines (N = 6). CXCL12 expression was positively but weakly correlated with the expression of CXCR4 or CXCR7. We also examined CXCL12 expression in 89 NSCLC specimens and found that CXCL12 expression was significantly higher in tumor specimens from female patients, non-smokers and adenocarcinoma patients. Small interfering RNAs targeting CXCL12 inhibited cellular proliferation, colony formation and migration of CXCL12-overexpressing lung cancer cells; however, this inhibition did not occur in lung cancer cells that lacked CXCL12. Furthermore, the anti-CXCL12 neutralizing antibody mediated inhibitory effects in three lung cancer cell lines that overexpressed CXCL12, but not in two CXCL12 non-expressing lung cancer cell lines nor two non-malignant bronchial epithelial cell lines. The present study demonstrates that: CXCL12 is concomitantly overexpressed with CXCR4 or CXCR7 in lung cancers; CXCL12 is highly expressed in NSCLCs from females, non-smokers and adenocarcinoma patients; and disruption of CXCL12 inhibits the growth and migration of lung cancer cells. Our findings indicate that CXCL12 is required for tumor growth and provide a rationale for the anti-CXCL12 treatment strategy in lung cancer.

IDENTIFICATION OF SARS-COV SPIKE PROTEIN-DERIVED AND HLA-A2-RESTRICTED HUMAN CTL EPITOPES BY USING A NEW MURAMYL DIPEPTIDE-DERIVATIVE ADJUVANT

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Severe acute respiratory syndrome (SARS) spread during the winter of 2003, and attempt have been made to develop vaccines against SARS corona virus (SARS-CoV). The present study provides a strategy to rapidly identify SARS-CoV-derived antigenic peptides recognized by HLA-A2-restricted cytotoxic T lymphocytes (CTLs). Forty-three candidate peptides having HLA-A2-binding motifs were selected in silico and HLA-A2/Dβ chimeric MHC class I-transgenic mice were immunized with these peptides and a new derivative of muramyl dipeptide that can induce upregulation of HLA-DR, CD80, CD86, and CD40 in human CD14+ antigen presenting cells, was administered as an adjuvant. Six HLA-A2-restricted mouse CTL epitopes were identified, including two new epitopes which have never been reported before. One of the novel peptides was naturally processed and successfully induced HLA-A2-restricted specific CTLs in both HLA transgenic mice and healthy donors. The method was useful, convenient and efficient for rapid identification of CTL epitopes derived from SARS-CoV proteins and will be possibly applicable for other pathogens to develop a peptide-based vaccine.
ORAL HYPOSENSITIZATION TO NICKEL INDUCES CLINICAL IMPROVEMENT AND A DECREASE IN TH1 AND TH2 CYTOKINES IN PATIENTS WITH SYSTEMIC NICKEL ALLERGY SYNDROME

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Some patients with nickel (Ni) allergic contact dermatitis suffer from systemic (intestinal or cutaneous) symptoms after ingestion of Ni-rich foods and experience symptoms reduction with low-Ni diet, a condition termed “systemic Ni allergy syndrome” (SNAS). We aimed at evaluating whether oral administration of low nickel doses improved clinical conditions and modulated immunological aspects of SNAS, without significant side effects. Thirty-six SNAS patients were enrolled. Treatment started after 1-month of low-Ni diet and consisted in an incremental oral NiOH dose phase (0.3 ng to 1.5 µg/week) followed by a 12-months maintenance phase (1.5 µg/week). Randomly, twenty-four patients added Ni therapy to low-Ni diet and 12 remained with diet alone. All patients were allowed rescue medications (antihistamines and topical steroids). After 4 months, Ni-rich foods were gradually reintroduced. In vitro allergen-driven IL13, IL5 and IFNγ release by peripheral blood mononuclear cells was evaluated before and after treatment. Twenty-three patients receiving NiOH and the 12 control patients completed the study. Evaluation of SNAS clinical severity (by VAS and drug consumption) showed a significant difference in favor of NiOH-treated patients compared to controls. Twenty of 23 patients in the NiOH group and none in the control group tolerated Ni-rich food reintroduction. Release of all studied cytokines in culture supernatants was significantly lower after NiOH treatment. In conclusion NiOH is effective in reducing symptoms and drug consumption in SNAS and is able to modulate inflammatory parameters.
NAD(P)H OXIDASE AND PRO-INFLAMMATORY RESPONSE DURING MAXIMAL EXERCISE: ROLE OF C242T POLYMORPHISM OF THE P22PHOX SUBUNIT

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Intense exercise induces a pro-inflammatory status through a mechanism involving the NAD(P)H oxidase system. We focused our attention on p22phox, a subunit of the NAD(P)H oxidase, and on its allelic polymorphism C242T, which is known to affect the functional activity of the enzyme. We investigated whether the p22phox C242T variants exhibit systemic effects in healthy subjects by analyzing the proinflammatory and cardiocirculatory responses to physical exercise in endurance athletes. The group of study consisted of 97 long distance runners, 37±4.4 yrs of age, with similar training history. The subjects underwent a maximal stress test during which both inflammatory and cardiopulmonary parameters were monitored. Our results demonstrate that T allele deeply influences the neutrophil activation in response to intense exercise, since T carriers were characterized by significantly lower release of myeloperoxidase (MPO), a classical leukocyte derived pro-inflammatory cytokine. In addition, the presence of T allele was associated with a higher cardiopulmonary efficiency as evidenced by a significantly lower Heart Rate (HR) at the peak of exercise and, when a dominant model was assumed, by a higher maximal oxygen uptake (VO2 max). On the other hand, no effects of 242T mutation on the plasmatic total antioxidant capacity (TAC) and on the cortisol responses to the physical exercise were detected. In conclusion, our data support a systemic role for p22phox C242T polymorphism that, modifying the intensity of the inflammatory response, can influence the cardiovascular adaptations elicited by aerobic training. These results contribute to support the hypothesis of a systemic effect for the C242T polymorphism and of its possible functional rebound in healthy subjects.
SOLUBLE CD30: A BIOMARKER FOR EVALUATING THE CLINICAL RISK VERSUS BENEFIT OF IFNβ1A TREATMENT IN MULTIPLE SCLEROSIS PATIENTS

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Aberrant redox regulation occurs in immune and neurological pathologies, hence targeting the pathways involved in the regulation of the redox system could provide further insights into these diseases and open up new avenues for therapy. Soluble (s) CD30 is of key clinical importance in this respect, as its levels reflect the functionality of the CD30 receptor (CD30R), the specific lymphocyte receptor for thiol disulfide/oxidoreductase thioredoxin 1 (Trx1) which is known to regulate important immune and neurological processes. Increased levels of sCD30 appear to be a common element of oxidative stress, immunological alterations and neurological deficit, therefore these increases could be used as a clinical biomarker and target for therapy. We targeted sCD30 in our study of dendritic cell (DC) regulation of the T helper (Th) cell network in multiple sclerosis (MS) patients, as abnormalities in T regulatory (Treg)/Th1/Th17 pathways contribute to the pathogenesis of this immunological/neurological disease. DC profiles in Treg/Th1/Th2/Th17-types of cytokine production in culture supernatants were used as they determine the type of Th differentiation. Our results show that sCD30 levels increase significantly in MS patients, reflecting the disruption in the regulation of the Treg/Th1/Th17 cell network. A fall in the level of soluble CD30, induced by IFNβ1a therapy, opposed the increase of neurological deficit through increasing IL10 and TGFβ levels, thus re-establishing network homeostasis but only when this was accompanied by an increase in IL12p70 levels. Since IL12p70 cytokine production is regulated by Trx1, our results indicate that redox system alterations may be the cause of IFNβ1a therapeutic inefficacy. We conclude that an increase in the level of IL10, TGFβ and IL12p70 and a fall in the level of sCD30 represent a means of evaluating the clinical risk/benefit of IFNβ1a treatment.

EFFECT OF TEMPERATURE ON THE SHIFT OF PSEUDOMONAS FLUORESCENS FROM AN ENVIRONMENTAL MICROORGANISM TO A POTENTIAL HUMAN PATHOGEN

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Pseudomonas fluorescens is a Gram-negative bacterium generally considered of scarce clinical significance. However, in the last few years, the isolation of P. fluorescens as causative agent of nosocomial infections has rapidly increased. P. fluorescens is a psychrophile microorganism which grows at an optimal temperature of 25-30°C. In spite of this constraint, it has recently been reported that the human physiological temperature does not appear to be a barrier for this microorganism. In this study we examined the ability of P. fluorescens, grown at 28°C or at 37°C, to adhere to cultured human A549 pulmonary cells and to form biofilm. The ability of P. fluorescens to induce expression of proinflammatory cytokines, beta-defensin 2 and the intercellular adhesion molecule-1 was also investigated. Our results clearly indicate that inflammatory mediators are induced when the microorganism is grown at a lower temperature, while biofilm is formed only at 37°C. The results presented are consistent with previous reports indicating P. fluorescens as an opportunistic pathogen and underscore the urgent need for further studies to better characterize the virulence of this microorganism.
LIPID METABOLISM IMPAIRMENT IN HUMAN GLIOMAS: EXPRESSION OF PEROXISOMAL PROTEINS IN HUMAN GLIOMAS AT DIFFERENT GRADES OF MALIGNANCY


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Gliomas are histologically graded by cellularity, cytological atypia, necrosis, mitotic figures, and vascular proliferation, features associated with biologically aggressive behaviour. However, abundant evidence suggests the presence of unrecognized, clinically relevant subclasses of the diffuse gliomas, both in respect to their underlying molecular phenotype and their clinical response to therapy. It is well-known that patient prognosis and therapeutic decisions rely on accurate pathological grading. Recently, it was reported that human gliomas accumulate lipid droplets during progression, suggesting a lipid metabolism impairment. Considering the crucial role of peroxisomes in lipid metabolism, in the present work we studied the expression profiles of proteins either exclusively localized to peroxisomes, such as peroxin14 (PEX14), peroxisomal membrane protein 70Kda (PMP70), acyl-CoA oxidase, thiolase, or partially associated to peroxisomes such as Hydroxymethylglutaryl-CoA reductase (HMGCoA-red) and peroxisomal-related proteins, namely PPARα, in human glioma specimens at different grades of malignancy. Moreover, Nile red staining of lipid droplets, thin layer chromatography (TLC) and proton nuclear magnetic resonance spectroscopy (NMR) were carried out in order to correlate the biochemical results with the lipid content of tumor tissues. The results obtained indicate that correlating the malignancy grade with the expression of peroxisomal genes and proteins, may constitute a sensitive tool to highlight possible subtypes not recognized by the classical histological techniques.

B LYMPHOCYTE SUBSETS AND THEIR FUNCTIONAL ACTIVITY IN THE EARLY MONTHS OF LIFE


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In the present study we evaluated B-cell subsets and their functional development in 74 newborns from birth to 6 months of life. Moreover, we evaluated “natural antibody” production in vitro. The results documented a predominance of naïve B-lymphocytes at all time-points evaluated, decreasing from birth to 6 months (p=0.009). The percentages of CD27+IgD+ and CD27+IgD− memory B-cells were very low at birth and significantly increased only at 6 months (p=0.02 and p<0.001, respectively). We found a significant increase only in in vitro stimulated IgG production at 6 months as compared to birth (p<0.001). Moreover, a lower secretion of anti-Pn IgM antibodies up to 6 months of age, as compared to controls was observed. Our results underline that the susceptibility and severe course of infection in the neonate can be attributed, at least in part, to the lack of pre-existing immunological memory and competent adaptive immunity.
LONG-TERM ANTI-TNF-α TREATMENTS REVERSE THE ENDOTHELIAL DYSFUNCTION IN RHEUMATOID ARTHRITIS: THE BIOLOGICAL COHERENCE BETWEEN SYNOVIAL AND ENDOTHELIAL INFLAMMATION


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Rheumatoid arthritis (RA) is associated with an excess cardiovascular morbidity and mortality, related to systemic inflammation with endothelial dysfunction (ED) and impaired flow-mediated vasodilation (FMD). We assessed the FMD response to anti-TNF-α treatments in 28 RA patients, aged 49.8±15.3 years: an unpaired FMD was found in 66.7% of our cases and was restored after 6 weeks of anti-TNF-α treatment (13.5±5.3% vs 4.6±4.1%, p < 0.05). Twenty-five percent of the infliximab patients demonstrated a long term response, compared with 60% of etanercept and 100% of adalimumab patients, after 2 years (p<0.01). Infections (3 cases), myocardial ischemia (1 case) or loss of response (4 cases) were associated with a worsened FMD, restored by shifting to adalimumab. The present study confirms that ED is an RA systemic disease marker, responsive to anti-TNF-α treatment and sensitive to clinical events or to a loss of response, underlying the biological coherence between synovial and endothelial inflammation.

THE OSTEOPONTIN GENE +1239A/C SINGLE NUCLEOTIDE POLYMORPHISM IS ASSOCIATED WITH TYPE 1 DIABETES MELLITUS IN THE ITALIAN POPULATION

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Secreted phosphoprotein 1, also known as Osteopontin (Opn), is a proinflammatory cytokine involved in the TH1 response and is highly expressed in the islets and pancreatic lymph nodes of non-obese diabetic mice before the onset of diabetes. In humans, typing of the +1239A/C single nucleotide polymorphism (SNP) in the 3’UTR of the Opn gene (SPP1) showed that +1239C carriers displayed higher Opn serum levels than +1239A homozygotes and a higher risk of developing autoimmune/lymphoproliferative syndrome, multiple sclerosis, and systemic lupus erythematosus. The aim of this work is to evaluate whether +1239A/C is also associated with type 1 diabetes mellitus (T1DM). We typed +1239A/C in an initial cohort of 184 T1DM patients and 361 controls, and confirmed our data in a second cohort of 513 patients and 857 controls. In both cohorts, +1239C carriers displayed a significantly higher risk of T1DM than +1239A homozygotes (combined cohorts: OR=1.63, 95%CI: 1.34-1.97). Clinical analysis did not detect any differences between patients carrying or not +1239C in terms of gender distribution and age at T1DM diagnosis. These data suggest that SPP1 variants marked by +1239C are associated with T1DM development in the Italian population. The predisposing effect may depend on its effect on Opn levels.
UNDIFFERENTIATED CONNECTIVE TISSUE DISEASE - AN UNSOLVED PROBLEM: REVISION OF LITERATURE AND CASE STUDIES

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In clinical practice, patients with a range of signs and symptoms suggestive of connective tissue disease, but who do not fulfill the classification criteria for a defined disease are often found. This condition is defined as “Undifferentiated Connective Tissue Disease” (UCTD). Most of the authors consider UCTD as a distinct clinical entity, generally stable during follow-up. Despite this, no mutual agreement regarding criteria for its diagnosis has been reached. The clinical, serological, therapeutical and evolutional patterns of 41 patients initially diagnosed as having early UCTD during a 3-year follow-up are described in this study. At the end of the observational period, 21% of the enrolled patients, followed throughout the follow-up, demonstrated clinical evolution to a defined connective tissue disease (CTD), whereas 52% of the observed subjects maintained an undifferentiated profile with variable clinical findings and presenting a generally stable disease over time. The remaining patients showed clinical improvement or complete regression of the symptoms associated with normalization of the inflammatory indexes. The role of therapy in these different clinical courses is discussed.

A SPLIT-MOUTH STUDY ON MICROBIOLOGICAL PROFILE IN CLINICAL HEALTHY TEETH AND IMPLANTS RELATED TO KEY INFLAMMATORY MEDIATORS


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This split-mouth study investigated the correlation of the qualitative and quantitative bacterial composition in dental plaque around clinically healthy periodontal and peri-implant subgingival sites with the levels of selected pro- and anti-inflammatory cytokines and the inflammatory infiltrate in the soft tissue surrounding a healthy dental implant and natural tooth in the same patient. Nine patients, all in good health and non-smokers, were studied. All of the patients were highly motivated in terms of oral hygiene and had healthy natural teeth and at least one healthy implant. After three sessions of professional oral care, clinical parameters were recorded. A sample of subgingival plaque was harvested from the buccal side of the selected implants and teeth. The plaque samples were cultured to quantify the total microbiota and the number of obligate and facultative bacterial strains. Simultaneously, from the lingual/palatal aspect of the same implants and teeth the keratinized periodontal and peri-implant soft tissues were biopsied for cytokine expression and histomorphometric analysis. The tissue biopsies were halved: the real-time reverse transcriptase-polymerase chain reaction (PCR) was performed to detect active TNF-α, IL-1β, IL-8, and TGF-β2 and distribution, composition, quantification of inflammation were assessed in parallel. The patients harbored no periodontopathogens and the microbiological composition of the plaque taken from implant sites did not differ from that harvested from teeth. No significant differences were seen between implants and teeth for both pro- and anti-inflammatory cytokines. Even the histological examination showed no significant epithelial changes, although slight perivascular lymphocytic infiltration was seen in some biopsies.
CIRCADIAN VARIATIONS OF CORTISOL, MELATONIN AND LYMPHOCYTE SUBPOPULATIONS IN GERIATRIC AGE

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A number of age-related changes in the 24-hour hormonal and non-hormonal rhythms have been found in older human beings. Lymphocyte subpopulations present circadian variation of some of their subsets and this variation may influence magnitude and expression of the immune responses. Numerous interactions exist among the nervous, endocrine and immune systems, mediated by neurotransmitters, hormones and cytokines. The aim of this study is to evaluate circadian variations of some endocrine and immune factors in older adults. Cortisol and melatonin serum levels were measured and lymphocyte subpopulation analyses were performed on blood samples collected every four hours for 24 hours from ten healthy young and middle-aged subjects and from ten healthy elderly subjects. There was a statistically significant difference between the groups in the observed values of CD20 (higher in young and middle-aged subjects) and CD25 and DR+ T cells (higher in elderly subjects). In the group of young and middle-aged subjects a clear circadian rhythm was validated for the time-qualified changes of all the factors studied. In the group of elderly subjects a number of rhythms were absent or altered. The results of the current study show that aging is associated with enhanced responsiveness of T cell compartment and alterations of circadian rhythmicity.

EVOLUTION OF AND RISK FACTORS FOR PSYCHOLOGICAL DISTRESS IN PATIENTS WITH PSORIASIS: THE PSYCHAE STUDY

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Psychological distress (PD) is common in patients with psoriasis but little is known about its evolution. The aim of this study is to assess the evolution of PD in psoriasis. For this purpose, 1,505 psoriatic patients, who had been previously enrolled in the PSYCHAE study, an observational multicenter Italian study, were re-evaluated after 6 and 12 months. Minor and major PD were assessed using the General Health Questionnaire (GHQ) and Brief Symptoms Inventory (BSI) questionnaires and coping using Brief COPE questionnaire. Minor PD was present in 46% of patients but halved during the study. Female gender, surface area, topical steroids, methotrexate, self-distraction, venting and behavioral disengagement were risk factors for minor PD; cyclosporine and humor were protective. Major PD was present in 11% of patients and remained stable. Female gender, venting, religion, behavioral disengagement and emotional support were risk factors for major PD; instrumental support was protective. In conclusion, the results obtained suggest that major PD remained stable after 12 months and that coping was a predictor of its evolution.
THYMOMA-ASSOCIATED IMMUNODEFICIENCY: A SYNDROME CHARACTERIZED BY SEVERE ALTERATIONS IN NK, T AND B-CELLS AND PROGRESSIVE INCREASE IN NAÏVE CD8+ T CELLS

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Thymomas are rare tumours that sustain T-lymphopoiesis and trigger a variety of autoimmune diseases and immunodeficiencies, including a fatal hypogammaglobulinemia, namely Good’s Syndrome (GS). Due to its rarity, GS has been poorly investigated and immunological features, as well as pathogenetic mechanisms underlying this syndrome, are unclear. We studied 30 thymoma patients by performing an immunological assessment, including immunophenotype and analysis of T cell repertoire (TCR). Development of GS was characterized by a progressive decrease in B, CD4 T and NK lymphocytes. These alterations paired with accumulation of CD8+CD45RA+ T cells that showed a polyclonal repertoire without expansions of specific clonotypes. GS is defined as hypogammaglobulinemia with thymoma. Here, we show for the first time that this syndrome is characterized by a severe loss of CD4+, NK and B cells. Furthermore, the accumulation of CD8+CD45RA+ T lymphocytes parallels these changes; this accumulation may have a role in determining the disease and can be used to monitor clinical stages of immunodeficiency in thymoma.
IS HPV-DNA TESTING A USEFUL TOOL IN PREDICTING LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION OUTCOME? A RETROSPECTIVE LONGITUDINAL STUDY

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HPV-DNA testing has entered in clinical practice. Three important questions remain controversial: 1) which is the best HPV-DNA technology? 2) Which age group should be targeted? 3) Is HPV-DNA testing predictive of disease outcome? The answers to these queries represent the endpoints of this study. The population of this retrospective study consisted of 272 women, each one having: baseline cytological diagnosis of Low-grade Squamous Intraepithelial Lesion (LSIL); baseline HPV-DNA reports by Hybrid Capture 2 (HC2) and MY09/11 consensus primers PCR; follow-up duration over 3-years; cytological report of disease status at follow-up time. Firstly, we assessed the concordance and the performances of both HPV-DNA testing, then we correlated respectively HPV-DNA results and age of patients to disease outcome. DNA testing methods agreed in 83.4% of cases (K=0.66). Baseline HPV-DNA result was not significantly associated to disease outcome (p=0.06). Within HPV-DNA positive group, we found no evidence of correlation between age and LSIL prognosis (p=0.89). Confining the analysis to age-stratified HPV-DNA negative women, the differences were statistically significant (p=0.01). In conclusion, HPV-DNA testing gives no information about the real behaviour of cervical abnormalities. These findings suggest the demand for additive markers, reflecting the risk of progression, in prevention strategy and clinical approach.

ELECTROCHEMICAL BEHAVIOUR OF TITANIUM IN AMMINE AND STANNOUS FLUORIDE AND CHLOREXIDINE 0.2% MOUTHWASHES

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Titanium (Ti) is widely used in dentistry. Fluorides at acid pH could destabilize Ti oxide and make it susceptible to corrosion. The behaviour of IV grade machined Ti disks in 5 electrolytic solutions: Fusayama artificial saliva (Fas), ammine fluoride-stannous fluoride (Am-SnF2), 0.2% Chlorexidine (CHX) 0.20%, Fas with 20% Am-SnF2 and Fas with 20% CHX, was evaluated. Open circuit potential (Ecorr) was determined by immersing Ti disks for 24 hours in an electrochemical cell containing the solutions, potential changes were measured until a stable value was obtained. Examination by Scanning Electronic Microscope and Energy Dispersive X-ray Analysis were then performed. One way ANOVA analysis showed a significant difference of Ecorr values regarding the 5 solutions (p<0.001). The highest values were observed for Fas (-37.6 mV), intermediate for Am-SnF2 (-81mV) and lowest for CHX (-87.6mV). SEM analysis of disks after polarization curve in CHX showed a marked localized corrosion, while the other solutions showed no considerable corrosive action on Ti surface. When considering corrosive potential range in oral cavity, Ti had an excellent behaviour on both antiseptics evaluated. The results obtained in this study will enable us to recommend the use of Am-SnF2 mouthwash for patients with dental implants.
HUMAN PULMONARY DIROFILARIASIS: ONE MORE CASE IN GREECE SUGGESTS THAT DIROFILARIA IS A RATHER COMMON CAUSE OF COIN LESIONS IN THE LUNGS IN ENDEMIC AREAS OF EUROPE

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Herein we describe a case of a 52 year-old male from Greece who presented with a coin lesion in the right lung, which proved to be an infection from \textit{Dirofilaria immitis}. A careful review of the literature shows that, contrary to the common perception, humans may be frequently infected by Dirofilaria species. For this reason the authors suggest that in every case which presents with a coin lesion in the lung in endemic areas, dirofilariasis should always be considered, and excluded before any other intervention is decided.

TRANSIENT HYPOGAMMAGLOBULINEMIA OF INFANCY: INTRAVENOUS IMMUNOGLOBULIN AS FIRST LINE THERAPY

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IVIG (Intravenous immunoglobulin) have significantly improved the prognosis and the quality of life of immunodeficient patients and are routinely used as substitutive therapy. Transient hypogammaglobulinemia of infancy (THI) is a primary humoral immunodeficiency characterized by a transient IgG defect, but is not considered as a disease that justifies substitutive treatment and thus the use of IVIG as an alternative to antibiotic prophylaxis remains controversial also in symptomatic children. We treated 13 THI children severely symptomatic with IVIG (400mg/kg/every 3 weeks) for a limited period (2 or 3 months) and followed them for 1 to 3 years. During the follow-up, the frequency of overall infections decreased approximately tenfold (from 0.39 to 0.047 infection/month per child) and no severe infections were reported. Although this study lacks untreated controls, the results suggest that the observed clinical improvement is correlated to IVIG therapy. Furthermore, our study suggests that the infused IVIG have no long-term effect on endogenous IgG production and do not lengthen the immunodeficiency condition since all children produced a normal amount of specific IgG in response to vaccination carried out 5 months after the end of infusions. In conclusion, our results suggest that IVIG may stop the vicious circle of infection-immunodeficiency and should be considered as a first line therapy in highly symptomatic THI children.
AN UNCOMMON CASE OF SYSTEMIC MUCORMYCOSIS ASSOCIATED WITH SPINAL CORD INFARCTION IN A RECENTLY DIAGNOSED DIABETIC

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Mucormycosis is a relatively rare, opportunistic, invasive infection caused by various members of the Phycomycetes class [from Greek φυκ-, phyko- seaweed; having a plant body], an extensive taxonomy introduced in 1956 to enlarge the class of Zygomycetes. These filamentous fungi have a worldwide distribution and are capable of rapid growth and thermotolerance of human body temperature. Infection typically occurs in seriously compromised patients (i.e. diabetic ketoacidosis, hematologic malignancies, immunosuppressive disorders, end-stage renal disease, solid-organ or bone-marrow transplantation) and can be acute or fulminant, as well as indolent and chronic. In this paper we describe a case of cutaneous mucormycosis that occurred in a 54-year-old diabetic woman and evolved into a disseminated form, leading to an uncommon spinal cord infarction and consequent paraparesis. Our case suggests the importance of suspecting a mucormycosis infection in patients with decompensated diabetes mellitus, even without ketoacidosis.

CD4+CD25+Foxp3+ T REGULATORY CELLS ARE NOT INVOLVED IN ORAL DESENSITIZATION

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Oral tolerance has been related to generation of T regulatory cells (Treg) or clonal anergy/deletion, respectively by administering low and high doses of fed antigens. CD4+CD25+ regulatory T cell clones can be induced by the antigen in Peyer’s patches of animal models. We selected ten subjects (mean age: 89.4 ± 36.21 months; group A) with severe cow’s milk allergy. They underwent oral desensitization (OD) according to the current protocols. In six months they reached a tolerance of 50 ml of cow’s milk. CD4+CD25+Foxp3+ T(reg) blood levels were measured at the beginning of OD (A) and after 6 months (A’), but almost the same values were obtained: A = 0.36 ± 0.11%; A’= 0.59 ± 0.15%. These results were compared with a control group (C) of non-atopic children. Naturally outgrowing cow’s milk allergy can be related to high blood levels of CD4+CD25+Foxp3+ T(reg), as previously reported in children. On the other hand, a forced oral desensitization through a progressive intake of the antigenic food seems not to be related to an enhancement of CD4+CD25+Foxp3+ T(reg) levels in peripheral blood, making the role of long-lasting systemic immunologic changes unlikely.
This retrospective study was conducted on 193 patients treated in three Italian Psoriasis Units with the aim of evaluating the evolution of psoriasis severity and the safety of cyclosporin A (Sandimmun Neoral®) in moderate to severe psoriasis, at the regimens usually employed in common clinical practice. Cyclosporin A (CyA) was administered for a mean period of 14 months, the mean number of treatment courses was 1.6 (range 1-4), and the mean dosage ranged from 1.5 to 3.1 mg/kg/die. Ninety percent of patients obtained complete therapeutic success or clinical remission, defined as complete clearance of lesions or clearance of lesions with residual minor pigmentations respectively, when treated with CyA in monotherapy. The mean Psoriasis Area and Severity Index (PASI) decreased from 23.31 before CyA administration to 5.64 at the end of treatment. The clinician’s judgement on CyA tolerability was good/very good in 90% of cases. Adverse events occurred in 36% of patients, with hypertension being the most commonly reported (17.6%). The results of this study indicate that in the common clinical practice CyA in moderate to severe psoriasis is usually employed at low doses, resulting both safe and effective.

GIANT SCROTAL ELEPHANTIASIS: AN IDIOPATHIC CASE

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Scrotal elephantiasis is very rare disease in industrialized countries, where it is mainly due to surgery, irradiation or malignancies. It can be defined as idiopathic only when the possible congenital, infectious and compressive causes are excluded. We report a case of massive scrotal lymphoedema in an adult Caucasian patient, in Italy. He presented an extremely voluminous scrotal mass measuring 50 x 47 x 13 cm (weight 18 kg), which extended below his knees, invalidating all his daily activities. The patient was hospitalized in order to undergo to surgical treatment. Although genetic causes were searched and the possible role of infectious agents and compressive factors was evaluated, no etiology was ascertained. Histopathologic examination showed non-specific chronic inflammation, confirming the diagnosis of idiopathic elephantiasis. One year after surgical treatment, the patient is healthy without recurrence signs.
CLINICAL, SEROLOGIC AND INSTRUMENTAL DATA OF TEN PATIENTS AFFECTED BY SCLERODERMATOUS CHRONIC GRAFT VERSUS HOST DISEASE: SIMILARITIES AND DIFFERENCES IN RESPECT TO SYSTEMIC SCLEROSIS

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Chronic graft versus host disease (cGVHD), the most common late complication of allogeneic haematopoietic stem cell transplantation (HSCT), may present with sclerodermatous lesions resembling in some cases the cutaneous involvement of systemic sclerosis (SSc). Certain pathogenetic findings connect the two diseases. In this report we describe ten subjects affected by cGVHD who underwent the examinations routinely carried out to stage SSc patients. Demographic, clinical, serologic and instrumental data were recorded. These patients showed differences in appearance, extent and progression of the sclerodermatous lesions with greater involvement of the trunk and proximal part of the limbs than the extremities. In seven subjects ANA test was positive; scleroderma-associated autoantibodies were not detected in any of the cases. Moreover, typical organ involvement of SSc was not found. Only one patient developed Raynaud’s phenomenon after HSCT and only one patient demonstrated a nailfold videocapillaroscopic scleroderma pattern. Except for cutaneous involvement of cGVHD, that may resemble SSc, the clinical features of the two diseases are quite different, suggesting that the fibrotic process characterizing cGVHD and SSc has different etiologies and different initial pathobiologic events.

PERIPHERAL TH-17 CELLS IN CHILDREN WITH ALLERGIC RHINITIS: PRELIMINARY REPORT

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Th17 is a subset of T helper lymphocytes and exerts pro-inflammatory activities. Recently, it has been reported that serum IL-17 levels are high in the most severe patients with birch allergy studied both outside and during the pollen season. This study aims to compare the frequency of peripheral IL-17-producing T cells in children with allergic rhinitis and in healthy controls. Ten children with allergic rhinitis and 5 healthy non-allergic subjects were evaluated. Th17 were evaluated by intracellular staining in ex-vivo T cell compartment. Ex-vivo PBMNC evaluation showed that allergic patients had higher frequencies of IL-17 producing T cells, both concerning CD4+ and CD8+ cells. In particular, there is a subset co-expressing IL-17 and IFN-γ both for CD4+ and CD8+ cells. In conclusion, this preliminary study suggests a possible role of Th-17 cells in the response to allergens in children.
The main problem associated with artificial vascular devices is the risk of bacterial infections, mostly sustained by coagulase negative staphylococci or \textit{Staphylococcus aureus}. Many efforts have been made to identify materials refractory to bacterial adhesion. The aim of our study is to verify the antimicrobial properties of two kinds of vascular prosthesis to prevent early onset infections and the efficacy of the concomitant action of a systemic antibiotic treatment. Adult male Wistar rats were used. We subcutaneously implanted in four groups a silver-coated prosthesis fragment, and a rifampicin-soaked prosthesis fragment in the remaining four groups. We inoculated in the site of implant a high bacterial burden of \textit{S. aureus} in four groups and a low burden in the remaining groups. Systemic levofloxacin was administered for seven days in four groups representing the two kinds of prosthesis; after 21 days the rats were sacrificed, prosthesis fragments were sonicated and the corresponding supernatants were plated for bacterial counts. The rifampicin-soaked prostheses explanted from rats treated with levofloxacin were sterile, regardless of the bacterial inoculum. In other groups some prostheses were colonized. In the experimental rat model used, the action of local and systemic antibiotic treatment was able to reduce colonisation of artificial prostheses.