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# ABSTRACTS

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**What have we learnt from our babies and children immunologically?**

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Babies and children with recurrent and/or severe infections have provided the impetus to delineate their underlying immunological defects so as to improve their treatment and prognosis. Through study of these babies, we have learnt so much about immunology. Babies with primary immunodeficiencies are experiments of nature, producing the knock-out phenotypes of the genes concerned. In the past 15 years, near hundred of these primary deficiencies have their genetic defects delineated. Phagocytic defects ranging from neutropenia to defects of adhesion/signaling/intracellular killing/granules have their molecular defects identified, illuminating how phagocytes function. Likewise, T cell defects can involve cytokine receptors, signaling molecules, and MHC molecules, all are essential components for normal T cell function. Babies with these defects have demonstrated the key functions of these various molecules in our immune response.

Babies even without underlying disease are more susceptible to infections especially intracellular organisms, such as *Herpes simplex virus* and *Mycobacterium tuberculosis*. These clinical issues led to study of neonatal T cell and monocyte/macrophage function to try to account for their susceptibility to infections.

Neonatal T cells are predominantly of naïve phenotype (CD45RA) as compared to adults (CD45R0). Their capacity of interferon-gamma, IL-4 and IL-12 production is lower than that of adults. We recently reported that insulin-like growth factor 1 can promote the maturation of neonatal naïve T cells to memory T cells as well as increase their interferon-gamma production to adult level (*Pediatr Res* 1999; 46:748-754; *J Immunol* 2000; 165:1331-1336).

Neonatal monocytes are less mature in their phenotypes and of less capacity in their production of IL-1 and tumour necrosis factor. We have recently investigated their capacity in differentiating into dendritic cells which are the key antigen presenting cells and found important differences from adult in terms of CD14, CD1a and mannose receptor expression, antigen capture and presentation ability. (*Brit J Haematol* 2001; 113:240-6; *Pediatr Res* 2001; 50:184-9) The cytokine production profile of T cells primed by DCs differ in IFN-γ and IL-10 depending on the combination of cord blood or adult blood T/DCs.

Study of such developmental immunology of babies can shed light on novel strategy to improve vaccine design, such as the conjugate vaccine in turning a T-independent vaccine to T-dependent one, as well as on mechanism of GvHD which is less frequent for cord blood transplant.

**Protective effect of melatonin on oxidative damage in burned rats**

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Burn injury is often accompanied by extensive release of free radicals, which cause oxidative damage in remote organs and are responsible for burn shock and multi-organ dysfunction. Melatonin has been considered as a very effective antioxidant in many animal models by detoxifying hydroxyl radicals and stimulating some antioxidative enzymes. Our study aims to observe whether melatonin also exerts a protective effect in burned rats.

Thirty male Sprague-Dawley rats (250-300g) were randomly divided into three groups: sham control; burn control and burn + melatonin (10 mg/kg, given immediately postburn by intraperitoneal injection). All animals were sacrificed 6 h postburn. A 30 % full-thickness burn was induced by the standard Walker-Mason method. Malondialdehyde (MDA, an indicator of lipid peroxidation), glutathione peroxidase (GSH-Px) and myeloperoxidase (MPO, an indicator of neutrophil infiltration) were assayed as described by Ohkawa *et al.* (for MDA), Flohe *et al.*(for GSH-Px) and Cetinkale *et al.* (for MPO). All data were expressed as Mean ± SE. One-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple range test were used for statistical analysis.

MDA levels in the lung and kidney of burn control showed a 39% and 31% increase respectively ($P<0.001$) compared to that in the sham control. After melatonin treatment, the MDA levels in these two organs decreased significantly and reached similar levels as the sham group. The MDA level in the liver didn't change in any of the three groups. GSH-Px activity showed a 30.3% decrease in kidney ($p<0.001$) with no significant change in the lung and liver. Melatonin treatment increased liver GSH-Px activity by 52% ($p<0.001$) compared with the burn control and had no effect in lung and kidney. MPO activity increased five-fold in the lung, two-fold in the liver and fourfold in the kidney 6 h postburn compared to that in the sham control. Melatonin treatment was found to be effective in decreasing MPO activity in all three organs.

Our study confirmed that major burn could cause significant oxidative damage in remote organs (lung and kidney) as evidenced by increased MDA levels. The study also found that neutrophil infiltration was the main source of free radicals released after burn injury. The results suggested that melatonin had a protective effect on organ oxidative damage in this 30% burn model. This protective effect might be mediated by direct scavenging of free radicals or by decreasing neutrophil infiltration and thus decreasing the release of free radicals.

**Primary immunodeficiency in adults**

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Immunodeficiency may occur primarily or secondarily to disease, infection or use of immunosuppressant. Primary immunodeficiency is usually presented in infancy or childhood but those of adult onset are not uncommon. Immunodeficiency may occur as a result of isolated or combined defects in T and B lymphocyte leading to impaired cell-mediated and humoral immunity. Immunodeficiency may also occur as a result of defects in phagocytic function. Common primary immunodeficiency syndromes of adult onset include common variable immunodeficiency (CVID), isolated IgA deficiency, idiopathic CD4 lymphopenia and chronic granulomatous disease. Patients with isolated B-cell defect have agammaglobulinaemia and are susceptible to bacterial infection. Patients with T-cell defect have impaired antigen stimulated proliferation, impaired delayed hypersensitivity response which is associated with significant peripheral lymphopenia. The impaired T-cell function is also commonly associated with secondary B-cell dysfunction because of lack of T-cell help. These patients are susceptible to viral, fungal and protozoal infection. Patients with immunodeficiency have significant morbidities and mortality. They also have greater chance of developing bronchiectasis, autoimmune disease or haematological malignancies. Regular intravenous infusion of immunoglobulin is required to maintain immunoglobulin levels sufficiency for immunity against various pathogens in patients with isolated B cell defect.

**Effects of swine bile acids-sodium salt and all-trans retinoic acid on human promyelocytic leukemia cell line HL-60**

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Swine bile acids-sodium salt (SBANa), the main component of artificial bezoar, is being used to treat acute gastroenteritis, dysenteric diarrhoea, whooping cough, acute infectious hepatitis, chronic tracheitis and simple dyspepsia. Recently we found that SBANa could inhibit the proliferation and induce differentiation of HL-60 cells. All-trans retinoic acid (ATRA) is commonly used to treat acute promyelocytic leukemia (APL). However, the patients often cannot bear the side effects (for example: thirsty, skin rash, enhancement of the activity of amino-transferase) of ATRA at the pharmacological dosage.

In this study we demonstrated that swine bile acids-sodium salt (SBANa) could act synergistically with ATRA at their sub-optimal concentration to inhibit the proliferation and induce the terminal differentiation of human promyelocytic leukemia cell line HL-60 cells. The HL-60 cells treated with sub-optimal concentration of SBANa and ATRA demonstrated mature macrophage morphology. The differentiated HL-60 cells also showed a high level of the activities of both α-NAE (α-naphthyl acetate esterase) and ACP (acid phosphatase). FACS analysis revealed that the treated cells were arrested at $G_0+G_1$ phase of the cell cycle. Moreover, the treated cells have more obvious NBT- reduction abilities and TPA-stimulated respiratory burst function.

Therefore, SBANa and ATRA can be used to treat the APL in a much lower concentration that still effectively inhibit the proliferation and induced terminal differentiation of the leukemia cells and minimized the side effects caused by the drugs.

**The anti-angiogenic effect of Sinomenine**

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Sinomenine (7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmorphinane-6-one), an alkaloid purified from the Chinese herb *Sinomenium acutum,* has a chemical structure similar to morphine. Sinomenine possesses several pharmacological activities, such as anti-inflammatory, analgesic, immunosuppressive and anti-arrhythmia. The application of *Sinomenium acutum* for the treatment of rheumatic diseases has been used in China for over two thousand years. The formulation using sinomenine as an active ingredient of *Sinomenium acutum* is known to be highly effective in treating rheumatoid arthritis and related diseases.

Angiogenesis is a fundamental process by which new blood vessels are formed. It occurs during embryonic development, endometrial regeneration and wound repair. The process involves proliferation, migration and morphological changes of the endothelial cells. In a healthy individual, the growth of vessel is under stringent control. However, rheumatic diseases appear to be driven by persistent, unregulated angiogenesis.

The anti-angiogenic effect of sinomenine was examined using human umbilical vein endothelial cell (HUVEC). The viability and the formation of capillaries like structure on Matrigel are not affected. However, Sinomenine inhibits not only the proliferation of HUVEC and reduces basic fibroblast growth factor (bFGF)- induced chemotaxis, but also the bFGF induced angiogenesis in the *in vivo* Matrigel plug assay. Results from our study suggest that sinomenine may be a natural anti-angiogenic compound.

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**The roles of NF-κB and P38 MAPK for the TNF-regulated ICAM-1 expression on human blood eosinophils and eosinophilic leukemia EoL-1 cells**

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Accumulation of eosinophils at inflammatory sites is mediated by selective adhesion and migration in allergic inflammation. The pro-inflammatory cytokine, tumor necrosis factor-α, TNF-α, has been shown to stimulate airway epithelial cells to produce cytokines and increases the expression of the intercellular adhesion molecule-1, ICAM-1. Nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPK) signal transduction pathway have been found to play significant role in recruitment of leukocytes to site of inflammation. To elucidate the intracellular mechanisms, we studied the NF-κB and MAPK signaling pathways through which TNF-α regulates ICAM-1 expression on eosinophils and EoL-1 cells *in vitro*. In this study, purified eosinophils from human buffy coat and EoL-1 cells were cultured with or without specific proteasome inhibitor N-cbz-Leu-Leu-leucinal (MG-132) and p38 MAPK inhibitor SB 203580, followed by TNF-α. The surface expression of ICAM-1 was assessed by immunocytochemical staining and the phosphorylation of p38 MAPK was detected by Western blot. NF-κB pathway-related genes were evaluated by cDNA expression array system whereas the activity of NF-κB was accessed by electrophoretic mobility shift assay (EMSA). TNF-α was found to up-regulate the surface expression of ICAM-1 in both blood eosinophils and EoL-1 cells. The gene expression of ICAM-1, NF-κB and IkBα of EoL-1 cells were also up-regulated. Furthermore, TNF-α was shown to induce phosphorylation of p38 MAPK. Moreover, TNF-α induced surface expression of ICAM-1 on EoL-1 cells was suppressed by MG 132, but not SB 203580. The result of EMSA indicated that MG-132 but not SB 203580 suppressed the TNF-α induced activation of NF-κB. In conclusion, NF-κB, but not p38 MAPK regulates the expression of ICAM-1, and subsequent adhesion and recruitment of eosinophils.

**Typhoid serology: the TUBEX® test inexplicably detects IgM but not IgG antibodies**

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Serological tests have played, and will continue to play, an important role in the laboratory diagnosis of typhoid fever since the time of Widal in 1896, while the Widal test has continued to be the most widely used. This test is popular because it is user-friendly (being one-step and requiring no instrumentation) and affordable even in poor countries. More accurate and sophisticated tests have been invented over the years but they are, generally, less user-friendly or affordable. These are based on the use of purified antigens, such as LPS and the flagellar or somatic protein antigens, in various methods best exemplified by the ELISA and immunoblotting. We recently introduced a new test that is both simple (one-step) and rapid (2 mins), in which the result is also easy to read (based on colour development). This is the TUBEX®, which uses coloured latex particles coated with a monoclonal antibody specific for the O9 antigen, and magnetic particles coated with LPS containing the O9 antigen, both contained in specially-designed reaction tubes. The test detects O9-specific antibodies from patients by their ability to inhibit the interaction between the two types of particles. The assay is adapted from an inhibition ELISA devised previously. Interestingly, unlike the inhibition ELISA, TUBEX® does not detect IgG antibodies, but only IgM. This unusual feature will be presented, and the underlying molecular mechanism, as well as the clinical importance, discussed.

**Identification of immunomodulatory compounds isolated from the Chinese medicinal herbs Banlangen**

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Banlangen (e.g. *Strobilanthes cusia, Isatis indigotica*) has been used to treat viral diseases for a long time in Mainland China. In this study, major components from the medicinal herbs *Strobilanthes cusia and Isatis indigotica* were evaluated for the antiviral and immunomodulatory activities. Several compounds were found to reduce the cytopathic effect of the influenza virus A/NWS/33 on MDCK cells. These include the partially purified polysaccharide fractions (MLG) isolated from the root of *Strobilanthes cusia*, purified compounds SLY-1, SLY-8, and the partially purified compound SLY-4 from the leaves of *Isatis indigotica*. RT-PCR was used to determine the effects of these compounds on chemokine expression. One of the compounds was found to reduce the expression of the chemokine RANTES. Our data suggest that the anti-viral activities of Banlangen may be related to the anti-viral and anti-inflammatory effects of these compounds.

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**Mast cells cultured from human peripheral blood**

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Mast cells are widely distributed thorough the body and are pivotal in the pathogenesis of inflammation and allergy. With reference to the recently reported method by Saito *et al.* (2001), we were able to culture homogeneous population of human mast cells from $CD34^+$ progenitor cells isolated from human peripheral blood. In general, $CD34^+$ progenitor cells were first cultured in a serum free medium containing 0.9% methylcellulose, 200 ng/ml SCF, 50 ng/ml IL-6 for 6 weeks with 5 ng/ml IL-3 added for the first 2 weeks. Colonies of mast cells grown on methylcellulose were then transferred to liquid culture medium containing the same concentration of SCF and IL-6 supplemented with 10% foetal calf serum for 4 more weeks before being characterized in the current study. These mast cells contained abundant granules in their cytoplasm which were stained positively for tryptase but not for chymase when characterised by immunostaining with antibodies against human mast cell tryptase and chymase. Hence morphologically, these culture human mast cells are similar to the tryptase containing mast cells ($MC_T$) isolated from human lung parenchyma and intestinal mucosa. Functionally these cultured mast cells responded to anti-human-IgE in a dose-dependent manner and 1/100 dilution of anti-IgE induced around 40% of histamine release after sensitisation with human IgE. Histamine release from these cells could also be induced by calcium ionophores (up to 50% at 1 μM of ionomycin or A23187) and high concentrations of compound 48/80 (around 10% at 1 μg/ml) and substance P (around 20% at 100 μM). Taking together, the mast cells we have cultured from human peripheral blood are phenotypically similar to the $MC_T$ subtype of human mast cell with functional characteristics similar to the airway epithelial mast cells retrieved from bronchoalveolar lavage fluid as described by Forsythe *et al* (2000).

H. Saito *et al.* (2001), *Int. Arch. Allergy Immunol.* 124:301-303
P. Forsythe *et al.* (1999), *Clin. Exp. Allergy* 30:225-232

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**Modulatory effects of the green tea polyphenol epigallocatechin gallate (EGCG) on the proliferation and differentiation of the murine hematopoietic cells and myeloid leukemia cells**

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Green tea is a widely consumed beverage in Asian countries, especially in China and Japan. Both epidemiological and experimental studies have shown that consumption of green tea can reduce the incidence of certain types of cancer in humans and experimental animals. Based on numerous studies, the biological responses of green tea are believed to be mediated by its major polyphenolic constituent, epigallocatechin gallate (EGCG). EGCG has been studied extensively due to its diverse physiological and pharmacological properties, including hypolipidemic, anti-inflammatory, anti-microbial, anti-oxidative, anti-carcinogenic and anti-tumor activities. In the present study, the modulatory effects of EGCG on hematopoietic cells and myeloid leukemia cells were investigated. We found that EGCG did not exhibit any significant cytotoxicity on the mouse spleen cells and bone marrow cells. Interestingly, exposure of the mouse bone marrow cells to EGCG for 72 h significantly enhanced their proliferation and differentiation *in vitro*. Apart from the normal hematopoietic cells, the anti-tumor effects of EGCG on myeloid leukemia cells were also studied. It was found that EGCG exhibited potent growth-inhibitory effect on the human promyelocytic leukemia HL-60 cells and the murine myelomonocytic leukemia WEHI-3B JCS cells. However, exposure of both cell lines to EGCG failed to trigger cellular differentiation, yet the leukemia cells were induced to undergo apoptotic cell death. Mechanistic studies indicated that the anti-proliferative and apoptosis-inducing effects of EGCG on HL-60 and JCS cells were mediated through the second messenger protein kinase C. Using RT-PCR, we showed that PKC-α and -δ were up-regulated by treatment with EGCG while the expression of PKC-β and -ε remained relatively constant. Taken together, our results suggest that EGCG may exert its anti-leukemic effect through its growth-inhibitory and apoptosis-inducing activities. On the other hand, it might have beneficial effects by restoration of the normal hematopoietic process after chemotherapy or radiotherapy.

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**Immunotherapy for allergic diseases**

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The prevalence of allergic diseases, including allergic rhinitis, asthma and atopic dermatitis, is increasing worldwide. This may be a result of lifestyle changes that increase the likelihood of allergic sensitization. Factors that influence allergic sensitization during early life include increased exposure to indoor allergens, reduced exposure to infections and the pattern of infant feeding. It has been shown in epidemiological studies that exposure to certain infectious agents or microbial products can actually skew the immune system away from a Th2 response, thereby reducing allergic sensitization. Improved living standards may therefore be responsible for the rise in incidence of allergies. Up until now, treatment of allergic diseases depends on blocking the effects of inflammatory mediators or the use of corticosteroids. These treatments do not offer a long-term solution to the problem. Allergen immunotherapy (IT) has in fact been in use for many decades. Modern techniques allow for better allergen standardization, thereby improving the efficacy and safety.

We are now beginning to understand the mechanism of action of allergen IT. Injection IT blunts the seasonal rise in specific IgE, and induces immunoglobulin class switching from IgE to $IgG_4$. There is probably also a shift from a Th2 profile to a Tr1 (IL-10) or Th3 (TGF-β) profile. There is evidence to suggest a reduction in T cell IL-4 and IL-13 expression after IT.

Recent studies have shown IT to have significant efficacy in seasonal and perennial allergic rhinitis against allergens such as pollens, dust mites and cat. Meta-analysis of clinical trials for asthma also showed efficacy. IT is the first line treatment for certain allergic disorders such as insect sting anaphylaxis. Long-term follow-up studies showed that clinical efficacy remains for at least three years after discontinuation of treatment. A very recent study also suggests that treatment of young children with allergic rhinitis can substantially reduce the risk of asthma in subsequent years. The potential adverse effects of IT such as local and systemic allergic reactions are being addressed by novel methods of treatment including sublingual IT, peptide IT and DNA vaccination. Allergen IT is a valuable treatment option in our fight against allergies and its potential role in disease prevention should be further explored.

**Studies on the anti-tumor activity of coumarins and their action mechanisms on myeloid leukemia cells**

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Coumarins belong to a diverse group of natural-occurring compounds known as benzo-α-pyrones. They can be isolated from a number of plant species, including *Melilotus officinalis*, *Prunus mahaleb* L., and orchids etc. A high content of coumarins is also present in our foods, such as soybeans and cruciferous vegetables. The derivatives of coumarin have different branch chains on the benzo-α-pyrone structure, and they can exist either in the free or glycoside form. Coumarin and its derivatives are phytochemicals with pleiotropic biological activities, including anti-edematous and anti-inflammatory activities, as well as immunostimulating and anti-carcinogenic activities. The immunomodulating activity of coumarins is possibly targeted to the monocyte/macrophages. Although there are increasing evidence showing that coumarins can exhibit anti-tumor activity *in vitro* and *in vivo*, however, their anti-tumor mechanisms remain poorly understood.

In the present study, the growth-inhibitory and cytotoxic effects of coumarin and its derivatives (7-hydroxycoumarin, esculetin and esculin) on different tumor cell lines were investigated. Our *in vitro* studies showed that coumarin and its derivatives inhibited the proliferation of various leukemias and a neuroblastoma cell line in a dose- and time-dependent manner. However, the cytotoxicity of coumarins on the normal mouse macrophages is relatively low. The anti-proliferative activity of coumarins is probably acting through the cell cycle arrest in the $G_0/G_1$ phase. Also, some coumarins can induce the differentiation of the human promyelocytic leukemia HL-60 cells and the neuroblastoma BU-1 cells, as measured by the morphological assay and confocal microscopy. Moreover, it was found that coumarins have apoptosis-inducing ability on myeloid leukemia cells in a time- and dose-dependent manner. The apoptotic pathways that are triggered by coumarins in myeloid leukemia cells were studied by the RT-PCR and Western blotting techniques. Mechanistic studies indicated that the apoptosis-inducing effect of coumarins on the murine myelomonocytic leukemia WEHI-3B JCS cells was possibly mediated through mitochondrial membrane depolarization and Fas/FasL activation. Further studies on the action mechanisms of coumarins and their effects on cell cycle progression in myeloid leukemia cells are currently in progress.

**Mitochondrial targeting drugs oligomycin and lonidamine (LND) bypassed the drug resistance and induced apoptosis in HepG2 Cells**

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Mitochondria are intimately involved in the delicate processes that sustain the balance between the life and death at the cellular level. Now, it becomes clear that mitochondria function not only as energy houses in eukaryotic cells, but also key regulators in apoptosis. Recently, there are increased interests for mitochondria as putative targets to kill cancer cells in chemotherapy. Here we provide evidence to support this therapeutic design with the use of oligomycin, an F1-Fo ATPase inhibitor, and lonidamine(LND), a new drug that selectively inhibits mitochondrial energy metabolism to treat cancer cells with multidrug resistance (MDR). A human hepatocelluar carcinoma cell line HepG2 and its derivatives with MDR phenotype (R-HepG2) were chosen to examine the effect of oligomycin and LND on cellular uptake of DOX, cytotoxicity, mitochondrial potential, apoptosis as well as cellular ATP level. Results in our study showed that P-glycoprotein (Pgp) was highly expressed in R-HepG2 but not in DOX-sensitive HepG2, which was consistent to the lower DOX uptake in R-HepG2 cells. Moreover, a higher rate of ATP production was detected in R-HepG2 when compared to that of HepG2. Oligomycin and LND caused a dose-dependent cytotoxicity in both HepG2 and R-HepG2, and combined treatment of cells with DOX in the presence of oligomycin or LND greatly increased cytotoxicity when compared to that of the single treatment with oligomycin, LND or DOX. Combined treatment with DOX and oligomycin caused an increase in DOX uptake. However, similar result was not observed in cells with DOX and LND treatment although both drugs caused a decrease in ATP production. Furthermore, oligomycin and LND could induce apoptosis in both cell lines by reducing the mitochondrial potential in a dose and time dependent manner. Our results suggested that mitochondrial targeting drugs oligomycin and LND were effective in killing cancer with or without Pgp through apoptosis. Mechanism involved in the induction of apoptosis awaits further investigation.

**Is mannose binding lectin (MBL) gene polymorphism predictive of damage in patients with systemic lupus erythematosus (SLE)?**

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Objective: MBL gene polymorphism leads to low serum levels of MBL. Previous studies have shown that MBL insufficiency predisposes to the development of rheumatoid arthritis and is associated with severe erosive disease. We hypothesized that MBL insufficiency in SLE is associated with severe disease and increased organ damage.

Methods: We aimed to determine the MBL genotypes and to measure MBL levels in SLE patients and to correlate them with clinical manifestations and damage according to the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index for SLE.

Results: 130 SLE patients with mean $\pm$ SD duration of disease of 9.2 $\pm$ 6.7 (median 7.0, range 1-31) years were recruited. MBL codon 54 gene mutation was detected in 40.1% (51/127) of patients and correlated with low serum MBL level ($p < 0.05$). 56.9% (74/130) patients had one or more SLICC/ACR score. The average SLICC/ACR score of these patients was 0.14 $\pm$ 0.26. However, there were no significant differences in damage scores between patients with various MBL phenotypes (levels above or below the median, $p = 0.71$) and genotypes (with or without codon 54 gene mutation, $p = 0.58$).

Conclusion: No relationship could be found between MBL insufficiency and SLICC/ACR score of any organ system in patients with SLE.

**Expression of cyclin B1 and cyclin-dependent kinase inhibitor p21 of lymphocytes in patients with systemic lupus erythematosus**

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Cyclins play an important role in controlling and regulating the cell cycle. The roles of cyclins and cyclin-dependent kinase (CDK) inhibitors of lymphocytes in the pathogenesis of systemic lupus erythematosus (SLE) are not fully understood. The objective of this study was to measure the expression of cyclin B1 and CDK inhibitor p21 in the peripheral blood lymphocytes from SLE patients and control subjects. Peripheral blood lymphocytes from 40 SLE patients with renal disease (RSLE), 40 SLE patients without renal disease (SLE) and 28 sex- and age-matched healthy control subjects were cultured in the presence of a T-cell specific mitogen, phytohemagglutinin (PHA). Bivariate distribution of expression of cyclin B1 or p21 versus cellular DNA content was assessed by flow cytometry. Expression of p21 in lymphocytes was significantly lower in RSLE and SLE patients than control subjects (RSLE versus controls, $p < 0.001$; SLE versus controls, $p < 0.001$). Expression of cyclin B1 was similar in all groups. The percentages of RSLE lymphocytes in G0/G1 and S phase were significantly reduced and elevated respectively, when compared with control subjects. Downregulated p21 in PHA-stimulated PBL from SLE patients may be closely related to the aberration of cell division in SLE lymphocytes.

**Mannose binding lectin (MBL) gene polymorphism and nephritis in patients with systemic lupus erythematosus (SLE)**

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Objective: MBL structurally resembles C1q and can activate the complement cascade via the third pathway. Deficiencies of both C1q and MBL have been shown to predispose to autoimmune diseases like SLE. Previous studies have shown that C1q is pathologically related to lupus nephritis. We hypothesized that MBL, like C1q is associated with proliferative lupus nephritis.

Methods: MBL genotypes and serum MBL levels were determined in SLE patients. Correlations between MBL genotype and phenotype in patients with history of renal involvement as defined as significant proteinuria $\geq$ 0.5 gm in 24 hours, and the different types of biopsy proven lupus nephritis (Class IV or V according to the WHO criteria) were studied.

Results: 130 SLE patients with mean $\pm$ SD duration of disease of 9.2 $\pm$ 6.7 (median 7.0, range 1-31) years were recruited. MBL codon 54 gene mutation was detected in 40.1% (51/127) of patients which correlated with low serum MBL level ($p < 0.05$). 42.3% (55/130) patients had history of renal involvement. Among them, 23 and 19 patients had biopsy proven Class IV and Class V nephritis respectively. Higher serum MBL was found to be associated with the development of Class IV lupus nephritis and at the level of 754.0 ng/ml (odds ratio = 6.3, 95% ci 2.0 – 9.8, $p = 0.002$). Only 8.7% (2/23) patients with Class IV lupus nephritis had MBL codon 54 gene mutation when compared with 45.2% (47/104) in those with no previous history of Class IV nephritis ($p = 0.001$). Serum MBL level was also found to correlate with the number of episodes of nephritis per patient-year of follow up ($r = 0.33$, $p = 0.01$).

Conclusion: Higher MBL levels were found in patients with Class IV nephritis suggesting a possible role of MBL, probably through immune-complex formation and deposition, in the underlying pathogenesis of proliferative lupus nephritis.

**Expression of GABA receptor subunit immunoreactivity in the rat subthalamic nucleus**

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Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter of neurons in the basal ganglia. Functions of GABA are mediated by $GABA_A$ receptors and $GABA_B$ receptors. Our recent reports have indicated that there is a differential localization of the two subunits of the metabotropic $GABA_B$ receptor, i.e., $GABA_BR1$ and $GABA_BR2$ receptors, in the rat neostriatum. In contrast, neurons in other regions of the rat basal ganglia, e.g., in the substantia nigra, are found to express both $GABA_BR1$ and $GABA_BR2$ subunits. In order to investigate the precise cellular localization of $GABA_BR1$ and $GABA_BR2$ receptor immunoreactivity in the rat subthalamic nucleus, perfuse-fixed sections of the rat subthalamic nucleus were used. The animals were deeply anesthetized with an overdose of sodium pentobarbital (60 mg/kg, i.p., Nembutal, Boehringer Mannheim) during perfusion. Double immunofluorescence was employed. Subthalamal neurons were labeled by using glutamate receptor GluR1 as specific neuronal marker. Results of the present study indicated that $GABA_BR1$ and $GABA_BR2$, and $GABA_A\alpha1$ immunoreactivity was found to display by subthalamal neurons that expressed GluR1 immunoreactivity. Intense $GABA_BR1$ and $GABA_A\alpha1$ labelings were found in the cytoplasm of the subthalmal neurons and in the neuropilar elements. In contrast, weak to moderate $GABA_BR2$ immunoreactivity was observed in subthalamal neurons. The present results together with our previous studies indicate that there are distinct patterns of localization of GABA receptors in the rat basal ganglia.

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***In vitro* studies on the mechanisms of hyperthermia- and TNF-α-induced apoptosis**

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Hyperthermia, being a potential treatment model for malignant diseases, has been applied in clinical trials. It is a procedure to elevate tissues temperature locally and artificially above the normal body temperature. However, up to now, the mode of action of hyperthermia is still far from clear. In this study, two cell lines, a tumour necrosis factor-α (TNF-α) sensitive L929 line and its derivative L929-11E cells with TNF-α resistance, were used to study the cytotoxic effect of hyperthermia and TNF-α. Interestingly, when both cell lines were challenged with hyperthermia (43°C, 1 to 3 hrs), apoptotic cells were observed. In parallel to this observation, (1) Mitochondrial membrane potential ($\Delta\Psi m$) was found to be reduced in a time-dependent manner after hyperthermia when measured by flow cytometry with JC-1; (2) A slow increase in the intracellular free calcium concentration ($[Ca^{2+}]_i$) accompanied with a decrease in $\Delta\Psi m$ was observed in cells challenged with hyperthermia as determined simultaneously by fluo-3 and TMRE; (3) $H_2O_2$ and the apoptosis-related proteins such as cytochrome c were released; (4) Poly (ADP-ribose) polymerase (PARP), pro-caspase-3 and bid protein were cleaved after hyperthermia treatment. These observations suggest that hyperthermia is able to induce apoptosis in L929 and L929-11E cells. It was also found that the cytotoxic effect exerted by hyperthermia was similar to that of TNF-α. Our results also indicate that the mitochondrial and post mitochondrial apoptotic pathways are functional in the L929-11E cells.

**Tumor necrosis factor-induced eotaxin release of human eosinophils is mediated by p38 mitogen-activated protein kinase and NF-κB**

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It has been shown that allergen-induced tumor necrosis factor (TNF) can increase eosinophil-specific chemokine eotaxin synthesis in eosinophils. Transcription factor nuclear factor-kappaB (NF-κB) and signaling molecule mitogen-activated protein kinases (MAPK) have been found to play a crucial role for the eotaxin-mediated eosinophilia. We investigated the activation of NF-κB and MAPK in TNF-induced eotaxin release of human eosinophils. TNF was found to increase the gene expression of NF-κB, its inhibitory protein IκBα and pro-inflammatory cytokines in eosinophils. TNF-induced IκBα degradation was suppressed by the proteasome inhibitor N-cbz-Leu-Leu-leucinal (MG-132) and a non-steroidal anti-inflammatory drug sodium salicylate (NaSal). Using electrophoretic mobility shift assay, both MG-132 and NaSal were found to suppress the TNF-induced NF-κB activation in eosinophils. Moreover, TNF was shown to induce phosphorylation of p38 MAPK time-dependently but not extracellular signal-regulated kinases (ERK). Inhibition of NF-κB activation and p38 MAPK activity could decrease the TNF-induced release of eotaxin from eosinophils. The above results indicate that NF-κB and p38 MAPK play a crucial role in TNF-activated signaling pathway regulating eotaxin release by eosinophils. It also provides a pharmacological basis for the potential of using specific inhibitors of NF-κB and p38 MAPK for treating allergic inflammation.

**CD14 expression in chronic periodontitis**

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Background: Chronic periodontitis is an opportunistic infection of the human tooth-supporting apparatus (periodontium) by gram-negative dental plaque bacteria leading to chronic inflammation and tissue destruction with or without granuloma formation of the periodontium, with eventual exfoliation of the teeth. The severity of periodontal disease is somewhat dependent on a dynamic equilibrium of interactions between the microbial challenge and host immuno-inflammatory responses. CD14 was described as a myeloid differentiation antigen detected on matured monocytes/ macrophages and neutrophils. It is a key molecule responsible for the innate recognition of bacteria LPS by these defense cells as a LPS or LPS/LBP complex receptor. AIM: The present study was to investigate the expression of CD14 in periodontal granulation tissues and inflamed gingival tissues from patients with chronic periodontitis.

Methods: Granulation tissues and inflamed gingival tissues biopsies were obtained from 11 patients with chronic periodontitis during periodontal surgery. The expression of CD14 was determined by immunohistochemistry staining.

Results: The expression of CD14 can be seen in both granulation tissues and inflamed gingival connective tissues, mainly on the surfaces of macrophages and neutrophils. In pocket epithelia of inflamed gingivae, CD14 was found and was confined at the region where dense infiltration of macrophages and neutrophils were found.

Conclusion: This study suggests that the expression of CD14 is associated with chronic periodontitis and may thus be involved in the host-mediated destruction of the periodontal tissues.

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**Distinguish the expression of different interferon-alpha subtypes in viral infection**

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Interferons (IFN) are induced by virus, double-stranded ribonucleic acid, antigen and mitogen. There are two distinct types of interferon families, type I and type II. Type I interferon consists of IFN-α, IFN-β and IFN- τ, which are produced by almost all cell types. The actions of type I interferon are less cell and tissue specific. Type II IFN, with only one member, IFN-γ, is primarily an immunoregulatory agent. IFN-α consists of many different sub-types. In human, IFN-α family consists of 13 different IFN-α genes all closely clustered on the short arm of chromosome 9 while the murine IFN-α family consists of at least 10 different genes clustered on chromosome 4. Although it has been demonstrated that some interferon alpha sub-types are more potent in combating virus, the role of each sub-type is poorly understood. To dissect the roles of different sub-types, a method to distinguish the sub-types is essential. Due to the great homology between IFN-α subtypes in gene and protein level, currently there is no biochemical or biological method to distinguish all the IFN-α subtypes. We have developed a set of specific primers that can distinguish the mRNA of different mouse IFN-α sub-types using RT-PCR method. The specificity of subtype specific primers was confirmed by the use of specific primers against different IFN-α subtype templates in RT-PCR. Using this set of specific primers and the RT-PCR method, we found that poly(I).poly(C) and different strains of influenza could differentially induced some IFN-α sub-types upon the mouse L929 cells.

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