ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

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Abstracts

ACTIVATION OF NF-κB BY TNF-α IN RAT OSTEOSARCOMA ROS17/2.8 CELLS

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Tumor necrosis factor-α (TNF-α) plays a key role in bone resorption and in inflammatory processes in bone and cartilage. In various tissues, TNF-α has been shown to exert its action mainly through an activation of a transcription factor, nuclear factor-kappa B (NF-κB). However, little is known how about TNF-α exerts its action in osteoblasts. In the present study, we examined the effect of TNF-α on the activation of NF-κB in rat osteoblast-like cells (ROS17/2.8) derived from osteosarcoma using electrophoretic mobility shift assay. Treatment of the cells with TNF-α induced the activation of NF-κB in the nucleus within 15 min. This induction continued for 48 h. In cytosol, NF-κB gradually decreased during the initial 60 min, suggesting that the activation of NF-κB immediately after TNF-α stimulation was mainly due to translocation of NF-κB from the cytoplasm to the nucleus. Characterization of NF-κB subunits using specific antibodies revealed that the NF-κB activated in the cells was p50-p65 heterodimer. These results for the first time showed that p50-p65 heterodimer NF-κB was immediately and persistently activated by TNF-α in ROS17/2.8 cells.

EFFECTS OF THYROID AND GLUCOCORTICOID HORMONES ON Kv1.5 POTASSIUM CHANNEL GENE EXPRESSION IN THE RAT LEFT VENTRICLE

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Effects of thyroid and glucocorticoid hormones on the expression of Kv1.5 potassium channel gene were studied in the rat left ventricle. Rats were rendered hypothyroidism by oral administration of methimazole. Hyperthyroidism was induced in the hypothyroid rats by administration of L-thyroxine. Kv1.5 mRNA level decreased markedly in the hypothyroid rats, whereas it increased in the hyperthyroid rats. The mRNA levels positively correlated with thyroid hormone levels in sera. When rats were adrenalectomized and were rendered hypothyroidism, Kv1.5 mRNA became undetectable. Administration of 3, 3′, 5-triiodothyronine (T3) at a dose to induce hyperthyroidism did not restore the mRNA level. However, T3 significantly increased the mRNA level when dexamethasone was co-administered at a physiological dose. These results for the first time demonstrate that thyroid hormone up-regulates Kv1.5 mRNA level in the rat left ventricle and that glucocorticoid is required for this induction.

NGFI-B, c-fos, AND c-jun mRNA EXPRESSION IN MOUSE BRAIN AFTER ACUTE CARBON MONOXIDE INTOXICATION

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The expression of immediate early genes (IEG) has been documented in the brain after various kinds of insults such as ischemia and hypoxia. To determine whether carbon monoxide intoxication (ACOI) might trigger IEG expression, adult ddY mice were subjected to a carbon monoxide intoxication at a rate of 30 ml/min for 35 sec. The levels of NGFI-B, c-fos, and c-jun mRNA were determined by Northern blot analysis. A time-course study in the cerebral cortex indicated that the induction of NGFI-B, c-fos, and c-jun mRNA started as early as 15 min, reached a peak 30 min, and returned to basal level at 1 hr after the ACOI. In addition, temporal feature of the induction of the IEG mRNA in the hippocampus was very similar to that in the cerebral cortex. Examination of brain regions at 30 min after the ACOI revealed a significant induction of NGFI-B mRNA in the cerebellum, thalamus-hypothalamus, brain stem, as well as in the cortex and hippocampus, but not in the striatum or olfactory bulb. Furthermore, the neuroanatomical distribution of c-fos mRNA at 30 min after the ACOI was found very similar to that of the NGFI-B mRNA. The widespread distribution of these IEG in the brain, especially in the cerebellum and brainstem, indicates that the main cause for the triggering of IEG expression in the brain by the ACOI might be a diffuse hypoxia. These findings show for the first time the temporal and spatial expression of IEG in the brain after ACOI.

SELECTIVE MATERNAL-ALLELE LOSS IN HUMAN LUNG CANCERS OF THE MATERNALLY EXPRESSED *P57*^{KIP2} GENE AT 11P15.5

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Genomic imprinting at 11p15 is suggested to play a role in certain pediatic tumors such as Wilms' rumor, based on the findings of selective maternal loss of this chromosomal region. Although the allele loss at 11p15 is also frequent in a number of cancers of adults including lung, breast, and bladder cancers, possible involvement of genomic imprinting in these tumors has not been invested extensively. *p57*^{KIP2}, a newly described member of the p21 cyckin-dependent kinase (CDK) inhibitor family which is thought to negatively regulate the cell cycle at the G1 checkpoint, has been mapped to 11p15. In the present study, we searched for somatic *p57*^{KIP2} mutations in lung cancer, but failed to find such alterations. Interestingly, however, we found that the *p57*^{KIP2} gene is imprinted with maternal expression and that the maternal alleles had been selectively lost in 11 of 13 (85%) lung cancer cases carrying 11p15 deletions, this being a significant bias (P=0.01). These data provide the first evidence that genomic imprinting may

play a role in the oncogenesis of not only rare pediatic tumors but also this common cancer of adults, suggesting that the imprinted $p57^{KIP2}$ CDK inhibitor gene is a potential target for maternally biased 11p15 deletions.

ANALYSIS OF ALLELIC STRUCTURES AT THE D7S21 (MS31A) LOCUS IN THE JAPANESE, USING MINISATELLITE VARIANT REPEAT MAPPING BY PCR (MVR-PCR)

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To sample the diversity of allelic structures at the D7S21 (MS31A) locus in the Japanese, allele-specific minisatellite variant repeat mapping using polymerase chain reaction (MVR-PCR) was performed on genomic DNA from a number of Japanese individuals. Three polymorphic positions in the MS31A 5′ flanking DNA were typed from 214 un-related Japanese, and the distribution of haplotypes was analysed. Allele-specific MVR-PCR, using primers that discriminate between different alleles at these polymorphic positions in heterozygous individuals, allows single alleles to be mapped from genomic DNA in approximately 80% of Japanese. 149 Japanese alleles have been mapped to date and all of them, except for one pair of indistinguishable alleles, have different internal structures. Heterozygosity was estimated at ~99.999% and more than 5000 different Japanese MS31A alleles must exist under Poisson analysis, assuming that all alleles are equally rare. More than half of the mapped alleles showed similar regions of internal structures to other alleles and were classified into groups on this basis. As well as being a potentially useful tool for forensic analysis, MVR-PCR analysis of MS31A alleles can be used to investigate allelic diversity, both within and between human populations, and to shed some light on possible mechanisms of minisatellite mutation.

ADHESION MOLECULES AND TGF-β1 ARE INVOLVED IN THE PERITONEAL DISSEMINATION OF NUGC-4 HUMAN GASTRIC CANCER CELLS

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Peritoneal dissemination is a common feature of recurrence after surgery in patients with gastric cancer. The presence of peritoneal metastasis after surgery is sure to affect the prognosis of patients with gastric cancer. Very little is known about the biochemical processes involved in the initial attachment of gastric cancer cells to peritoneal mesothelial cells. We conducted *in vitro*

and *in vivo* studies to assess the role of adhesion molecules and TGF-β1 in this process, using 4 cell lines derived from human gastric cancers. NUGC-4 cells, which disseminate early after inoculation into the abdominal cavity of nude mice, predominantly expressed CD44H and β_1 integrin. We found that NUGC-4 cells adhered to monolayers of mesothelial cells more firmly than the other cell lines. Adhesion of NUGC-4 cells to mesothelial cells was partially inhibited by antibodies against CD44H or the β_1 subunit of integrin, and was completely blocked by a combination of these 2 antibodies. Treatment with ligands for CD44H and β_1 integrin also inhibited this adhesion. In the cell culture medium of NUGC-4, large amounts of Transforming growth factor β1 (TGF-β1) was detected in proportion to the number of cancer cells, and they were more than in the other cell lines. TGF-β1 increased the expression of CD44H in NUGC-4 cells and in mesothelial cells, and augumented the adhesion and implantation of NUGC-4 cells to mesothelial cells accompanied by accumulation of extracellular matrix (ECM) components. Treatment by antibodies against both CD44H and β_1 integrin inhibited the dissemination of NUGC-4 cells in the peritoneal cavity of nude mice, and prolonged their survival time. These findings suggest that CD44H and integrins mediate the initial attachment of gastric cancer cells to mesothelial cells, and TGF-β1 participates in the promotion of the disease. Increases both in the expression of CD44H and in the amount of ligands for CD44H and integrins induced by TGF-β1 might promote the early development of peritoneal dissemination. It is possible that a treatment that interferes with the function of CD44H and integrins may result in the decreased intra-abdominal spread of gastric cancer.

ENHANCEMENT OF *IN VITRO* AND *IN VIVO* ANTI-TUMOR ACTIVITY OF ANTI-G_{D2} MONOCLONAL ANTIBODY 220-51 AGAINST HUMAN NEUROBLASTOMA BY GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND GRANULOCYTE COLONY-STIMULATING FACTOR

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We have evaluated the anti-tumor effect of anti-G_{D2} mouse monoclonal antibody (mAb) 220-51 against human neuroblastoma cell line TGW-I-nu *in vitro* and *in vivo*. The mAb 220-51 could mediate complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) using human effector cells. When mAb 220-51 was administered to tumor-bearing nude mice, tumor growth was significantly inhibited as compared with untreated controls. Furthermore, we have evaluated the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) on the anti-tumor effect of mAb. In the presence of recombinant human GM-CSF, granulocyte ADCC was significantly augmented *in vitro*. Systemic administration of recombinant murine GM-CSF in combination with mAb 220-51 significantly enhanced the anti-tumor effect of mAb *in vivo*. Recombinant human G-CSF combined with mAb 220-51 could also enhance it, although granulocyte ADCC was not affected by the presence of recombinant human G-CSF *in vitro*.

Moreover, GM-CSF and G-CSF work additively to enhance the anti-tumor effect of mAb 220-51 *in vivo*. In conclusion, GM-CSF and G-CSF may have a clinical potency in immunotherapy with anti-G_{D2} mAb for the treatment of neuroblastoma.

EFFECTS OF A NONAPEPTIDE THYMIC HORMONE ON INTESTINAL INTRAEPITHELIAL LYMPHOCYTES IN MICE FOLLOWING ADMINISTRATION OF 5-FLUOROURACIL

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A significant fraction of murine small intestinal intraepithelial lymphocytes (i-IELs) maturate in local sites outside the thymus. However, there is evidence suggesting that extrathymic differentiation of i-IELs is still influenced by the thymus or thymus-derived factors. Facteur thymique serique (FTS), a nonapeptide thymic hormone, is involved in several aspects of intra- and extrathymic T cell differentiation *in vivo*. In this study, we investigated the effects of FTS on the kinetics of i-IELs in mice following a single administration of 5-fluorouracil (5-FU). FTS treatment significantly accelerated the recovery in cell number of i-IELs after administration of 5-FU. Flow cytometric analysis revealed that this accelerated recovery was mainly due to a rapid increase in CD8$\alpha\alpha^+$ i-IELs. Similar findings were also evident in adult thymectomized (ATX) mice, indicating that FTS treatment caused a rapid recovery of CD8$\alpha\alpha^+$ i-IELs following 5-FU administration in the absence of a functional thymus. Furthermore, expression levels of the mRNAs for interleukin-2, interferon-γ and transforming growth factor-β_1 in the i-IELs were augmented by FTS treatment. Notably, FTS treatment protected mice from 5-FU-induced lethal toxicity, accompanied with an inhibition of the translocation of *Enterobactericeae*. These results suggest that FTS has an important function in the extrathymic maturation and activation of i-IELs in the small intestine following 5-FU administration, which may contribute at least partly to the protection against 5-FU-induced lethal toxicity.

FUNCTIONAL DIFFERENCES AMONG MULTIPLE ISOFORMS OF GUINEA PIG DECAY ACCELERATING FACTOR (DAF)

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Decay accelerating factor (DAF, CD55) is a membrane inhibitor, which protects host cells from the autologous C-mediated attack. The guinea pig homologue of DAF consists of multiple isoforms generated by alternative splicing from a single copy gene. These isoforms are mainly

comprised of a GPI-anchored form (GPI) and a transmembrane form (TM) which is not present in human DAF. Both forms occur in at least three variations which differ in the length of the Ser/Thr-rich region (termed ST-a, ST-ab, and ST-abc). We have transfected cDNAs of the six major isoforms into Chinese hamster ovary cells and their functional differences were evaluated in inhibition of C-mediated cytolysis and C3 deposition, using the transfectants expressing DAF at the same level on cell membranes. The degree of inhibition in both the classical and alternative pathways differed according to the length of the ST region in the order of abc > ab > a in both GPI and TM forms. When GPI and TM forms were compared, those with the ab or abc variation exhibited almost the same activity, whereas a-TM was less efficient than a-GPI. Although several isoforms are constitutively expressed in most of tissues, spermatozoa preferentially express the abc-GPI isoform suggesting that this isoform offers effective protection to spermatozoa in the female genital tract.

PROTECTIVE MECHANISMS AGAINST TUMOR NECROSIS FACTOR-ALFA-INDUCED LIVER INJURY BY DIBUTYRYL CYCLIC ADENOSINE MONOPHOSPHATE

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Cyclic adenosine monophosphate (cAMP) increasing agents are known to prevent endotoxin/TNF-α-induced liver injury. The mechanisms whereby dibutyryl cAMP (DBcAMP) protects D-galactosamine (D-gal) -sensitized mice from TNF-α-induced liver injury were examined. Liver injury was provoked by an intravenous injection with recombinant TNF-α (1.0 μg/kg) together with D-gal (500 mg/kg). Various doses of DBcAMP were injected intraperitoneally one hour before injection with D-gal/TNF-α. Acute liver damage, heat shock protein (HSP) 70 induction in hepatocytes and in vivo cytokine production were determined. DBcAMP protected from TNF-α-induced liver injury accompanied by apoptosis of hepatocytes. DBcAMP significantly enhanced expression of HSP70 in hepatocytes in close correlation with suppression of serum alanine aminotransferase levels after D-gal/TNF-α challenge. Furthermore, soluble TNF receptors type II (sTNFRII) neutralizing TNF-α were significantly increased but Interleukin-1β levels was decreased in the serum after challenge with D-gal/TNF-α in mice pretreated with DBcAMP. These results suggest that increments in HSP70 expression and sTNFRII production are involved in the protective effects of DBcAMP on TNF-α-induced liver injury in D-gal-sensitized mice.

EXPRESSION OF HEPATIC INDUCIBLE NOS AND ITS ROLE IN PHAGOCYTIC KILLING OF BACTERIA BY THE HEPATIC RETICULOENDOTHELIAL SYSTEM IN VIVO AND IN VITRO

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Phagocytosis and killing of circulating bacteria by the hepatic reticuloendothelial system (RES) are important for host defense, comprising ~80% of RES functions. Furthermore, Kupffer cell, 9 resident macrophage in the liver, may play a pivotal role in this system. Here, we investigated expression of *iNOS* in the whole liver and isolated Kupffer cells, and evaluated the putative role of *iNOS* in killing in a model that discriminatively quantitates hepatic phagocytic clearance and killing of *E. coli.* RNA was extracted after stimulation by endotoxin (LPS) from the liver and Kupffer cells, and analyzed by Northern blotting. *In vivo* experiments, rats were injected via femoral vein with 10^8 *E. coli,* double-labeled with ^{51}Cr and ^{125}I-UdR. In the livers harvested 30 minutes later, total hepatic activity of ^{51}Cr reflected the proportion of the total number of injected bacteria trapped (phagocytosed) by the hepatic RES (Hepatic Phagocytic Clearance = HPC), while the difference in activities of $[^{51}Cr] - [^{125}I]$ reflected the proportion of these bacteria that had been degraded (killed) (Hepatic Killing Efficiency = HKE). Net Hepatic Killing (NHK) reflects the product of HPC × HKE. This assay is validated by quantitative culture (CFU). Bacterial phagocytosis and killing were also quantitated using ^{51}Cr-labelled *E. coli* and CFU respectively *in vitro.* HPC was unaffected by these treatments. A prior intraperitoneal LPS injection significantly enhanced HKE from 20% up to 35% and consequently, NHK. Confirmed prior inhibition of *iNOS* (*N-mono-methyl-L-arginine*) ablated this enhancement of HKE (& NHK). This ablation was reversed by confirmed reversal of *iNOS* inhibition with L-arginine. *In vitro* experiment, same results were validated. Phagocytic killing of bacteria by the hepatic RES *in vivo* and *in vitro* is significantly potentiated by prior exposure to endotoxin via a mechanism dependent upon the synthesis of nitric oxide.

NITRIC OXIDE CONTROLS SRC KINASE ACTIVITY THROUGH COVALENT BOND-ORIENTED MOLECULAR MODIFICATION

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c-Src kinase was phosphorylated and activated when murine NIH3T3 fibroblast cells were incubated with nitric oxide generator, S-nitroso-N-acetyl penicillamine (SNAP) or sodium nitroprusside. c-Src kinase was also activated when SNAP was directly added to the enzyme isolated by immunoprecipitation. Nitric oxide scavengers hemoglobin and homocysteine abolished the SNAP-mediated activation of c-Src kinase, suggesting the involvement of nitric oxide as the

ultimate effector. Phosphoamino acid analysis and cyanogen bromide cleavage maping of autophosphorylated c-Src kinase showed that SNAP selectively promoted phosphorylation at tyrosine and the phosphorylation preferentially took place at tyrosine 416. Na_3VO_4 did not abolish the SNAP-mediated activation of Src kinase excluding the possible role of co-immunoprecipitated PTPase in the activation mechanism. v-Src kinase, constitutively activated for lacking of Y-527, was also further upregulated in activity by SNAP. This suggested that the SNAP-mediated activation of Src kinase was independent of the known Y527 phosphorylation/dephosphorylation-linked control. The V_{max} values of both c-Src and v-Src kinases were increased whereas K_m values were decreased after treating the enzyme with SNAP. The increased catalytic activity of the SNAP-treated Src kinase was abolished by treating the kinase with 2-mercapto ethanol (2-ME) or dithiothreitol, but was regained after re-exposing the enzyme to SNAP. Sulfhydryl reactive tyrosine kinase inhibitor herbimycin A blocked the SNAP-mediated activation of the enzyme suggesting the involvement of common target sites for both of the compounds. Exposure of Src kinase to SNAP promoted autophosphorylation and aggregation of the kinase molecules simultaneously, and both autophosphorylation and aggregation were prevented in the presence of 2-ME. Taken together, these results suggest a redox-linked covalent bond-mediated reversible mechanism for Src kinase activation which is independent of the known Y527 phosphorylation-linked regulation.

A NOVEL TRUNCATED VARIANT FORM OF Ebk/MDK1 RECEPTOR TYROSINE KINASE IS EXPRESSED IN EMBRYONIC MOUSE BRAIN

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We have isolated cDNA clones from a mouse embryonic head cDNA library that encode one member of the Eph/Eck family of receptor tyrosine kinases (RTKs), Ebk/MDK1. Among the 10 clones, two showed full-length type comprising extracellular, transmembrane and intracellular kinase domains. Two of them were modified just after the transmembrane domain and stop codon appeared before completing the kinase domain. This truncated form also had a deletion of five amino acids at the extracellular domain, indicating that it is a novel variant of Ebk/MDK1. RNase protection assay showed that this truncated deleted type, named Ebk-td1, is present in the head of embryos, although the amount is less compared to that of the full length type having a deletion of four amino acids. Considering the source and expression of Ebk/MDK1 mRNAs, they may play an important role, accompanied with a possible regulatory role of the truncated variant, during neural development and/or in embryogenesis.

ADENOSINE REVERSES HYPERGLYCEMIA-INDUCED INHIBITIONS OF PHOSPHOINOSITIDES SYNTHESIS IN RETINAL PIGMENT EPITHELIAL CELLS, AND NERVE CONDUCTION VELOCITY IN DIABETIC RATS

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The effect of 2-chloroadenosine (C-AD) on glucose-induced inhibition of phosphoinositide synthesis was studied in retinal pigment epithelial (RPE) cells, by monitoring the level of the phosphatidylinositol (PI)-synthase substrate, cytidine diphosphate diglyceride (CDP-DG). In high aldose reductase expressing RPE 91 cells, C-AD decreased CDP-DG at basal glucose level, and reversed the increase by 20 mM glucose. Adenosine deaminase, which inactivates endogenously released adenosine, potentiated hyperglycemia-induced increase in CDP-DG. C-AD failed to have any effect in low aldose reductase-expressing RPE 45 cells, but decreased CDP-DG in cells exposed to 300 mM glucose for 1 week, and switched back to media containing normal glucose. The mechanism of the adenosine-regulation on PI-synthase in cells with high aldose reductase activity is unknown, but it is independent of Gi or Gs proteins, adenylate cyclase and phospholipase C activation, myo-inositol-uptake or myo-inositol-efflux, or by direct regulation of PI-synthase. Administration of C-AD into streptozotocin-induced diabetic rats reversed the slowing of motor nerve conduction velocity. Thus adenosine-derivatives, which reverse a glucose-induced deficit in phosphoinositide metabolism might serve as a useful pharmacological tool to intervene hyperglycemia-induced diabetic complications.

CORTICAL HYPOMETABOLISM AND DELAYED MYELINATION IN WEST SYNDROME

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We examined the relation between cortical hypometabolism and delayed myelination in patients with West syndrome. Serial positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose and magnetic resonance imaging (MRI) were performed in 18 patients with West syndrome, first at the onset of epileptic spasms and later at 10 months of age. The age at onset of seizures ranged from 2 to 7 months. Ten patients were diagnosed as having cryptogenic West syndrome and 8 as symptomatic West syndrome. Cortical hypometabolism was detected in many patients at onset of epilepsy, but disappeared later, while delayed myelination tended to become evident with age. PET showed diffuse or focal cortical hypometabolism in 12 patients at onset, but only in 6 patients at 10 months of age. MRI revealed delayed myelination in only 2 patients at onset of epilepsy, but the number of patients with delayed myelination increased to 12 at the age of 10 months. Delayed myelination was more often present in patients with

cortical hypometabolism. Delayed myelination was seen in 11 (85%) of 13 patients with cortical hypometabolism on first or second PET scans, but in only one (20%) of 5 patients who did not show PET abnormalities. Hypometabolism on the first or second PET scan was positively correlated with delayed myelination at 10 months of age. In patients with West syndrome, it is important to assess myelination with MRI again at 8–10 month of age even when MRI at the onset appears normal. Serial MRI and PET reveal more detailed pathophysiology of West syndrome.

DEATH RISK OF CREATININE GENERATION RATE IN LONG-TERM HEMODIALYSIS PATIENTS

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Since the creatinine generation rate (CGR) correlates with the muscle mass, it may be a useful indicator for the protein nutritional status, which may be affected by numerous factors such as dietary habits, hemodialysis prescription and underlying diseases. If so, some of the factors associated with probability of death would impact the survival prognosis through a lowered protein nutritional status, which may be assessed by the level of the CGR. Thus, in 1,145 hemodialysis patients, the impact of various case mix and laboratory variables on the survival prognosis was evaluated using the logistic regression model with and without the percent CGR (i.e., %CGR: the percentage of the CGR of a patient in question relative to the mean value of that observed in non-diabetic patients of the same sex and age.), so as to compare the impact level of each variable between the two models.

By analyzing the model without %CGR, it was shown that advanced age, occurrence of diabetes, male sex, lower Kt/V, lower protein catabolic rate (PCR), the higher percent weight decrease during hemodialysis (the difference between the pre- and postdialysis weights divided by the postdialysis weight), and lower %CGR, were significant death risk factors. However, when %CGR was added to the model, the relative risk of death from occurrence of diabetes decreased greatly, and the risk from lower Kt/V and lower nPCR was no longer significant. These results suggest that at least part of the death risk from the occurrence of diabetes, lower Kt/V and lower nPCR may be due to the lower %CGR.

ALTERATION OF THE KINETICS OF TYPE I PROCOLLAGEN SYNTHESIS IN HUMAN OSTEOSARCOMA CELLS BY 1,25-DIHYDROXYVITAMIN D3

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The kinetics of type I procollagen synthesis in a human osteosarcoma cell line, MG 63, were investigated after treatment with 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2$ D_3), a hormonal inducer of phenotypic differentiation. Pulse label and chase experiments demonstrated greatly enhanced production and more rapid reduction of intracellular procollagen molecules in the 1,25-$(OH)_2$ D_3-treated cells as compared to the non-treated case. After a chase for 1 h, labeled procollagen was reduced by nine tenths in 1,25-$(OH)_2$ D_3 treated cells while half of the radioactivity still remained in non-treated cells. The expression rate of type I collagen, which was examined by pulse label experiment, was elevated in association with an increase in the mRNA coding for the type I collagen $\alpha 1$ chain by 1,25-$(OH)_2$ D_3 treatment. However, the amount of intracellular procollagen present after 4 h continuous labeling was almost the same, independent of the 1,25-$(OH)_2$ D_3 treatment. Thus we conclude that strage of the molecule was not affected. The results therefore suggest an increase in both the synthesis and secretion of type I collagen. The 1,25-$(OH)_2$ D_3 treatment was also found to induce the a subunit of prolyl 4-hydroxylase and to be associated with an elevated level of hydroxyproline in the procollagen. Moreover, gelatinase B-resistant procollagen molecules, indicative of intracellular procollagen molecules in the stable triple helical form, were only detected in the 1,25-$(OH)_2$ D_3 treated cells. These data suggest more efficient proline hydroxylation is involved in rapid secretion of procollagen after hormone administration. The present evidence points to post-translational control of procollagen synthesis.

EVALUATION OF HEPARIN-BINDING GROWTH FACTORS IN RESCUING MORPHOGENESIS OF HEPARITINASE-TREATED EMBRYONIC LUNG EXPLANTS

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In vitro development of embryonic mouse lung explants was hindered by digestion with heparitinase, when removed about 40% of [^{35}S] sulfate-labeled heparan sulfate synthesized. The enzyme-treated explants were inhibited in branching morphogenesis and the mesenchymal tissue was thin. Addition of basic fibroblast growth factor (bFGF), a typical heparin-binding growth factor, restored the inhibition caused by heparitinase in branching morphogenesis. Addition of midkine (MK), another heparin-binding growth factor, showed a weak effect on branching

morphogenesis, but exhibited an effect in restoring development of mesenchymal tissue. These data together with the distribution of the factors indicate that both are involved in development of the lung. Heparitinase-treated explants can be useful models for evaluating roles played by various heparin-binding growth factors.

TRANSPLANTATION OF CULTURED MUCOSAL EPITHELIUM: AN EXPERIMENTAL STUDY

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We investigated morphological changes after transplantation of cultured mucosal epithelium using modified *Barrandon*'s method (1988). Serially cultivated human mucosal epithelium was transplanted onto the back side of rectangular dorsal skin flap of nude mice. The morphological changes of the epithelium were observed by use of paraffin sections. The modified *Barrandon*'s method which was took place in this study has advantages such as minimum external trauma and less opportunity of infection. The cultured epithelium was taken within 1 week and gradually increased its epithelial thickness. Keratinized epithelium occurs after 3 weeks. Until 4 weeks after grafting, the grafted epithelium was consisted with 7 to 10 cell layers. The structure of transplanted tissue, in conjunction with surrounding connective tissues, showed dermis-like features until day 7 after transplantation. From these results, the cultured mucosal epithelium could be successfully transplanted and the morphology was similar to that of normal mucosal tissue.

STRUCTURAL CHANGES AND CELL VIABILITY OF CULTURED EPITHELIUM AFTER FREEZING STORAGE

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Numerous clinical reports have shown the utility of cultured epithelial grafting in the field of plastic and reconstructive surgery. Recently, freezing storage of the cultured epithelium has been tried and successfully grafted after thawing. It is clinically convenient if it is possible for cultured epithelium to keep its normal structure and viability. However, few papers have described the structural changes in cultured epithelium after freezing storage. In the present study, the morphological changes and cell viability of cultured mucosal epithelial sheets after freezing were observed in comparison with cultured epidermal sheets. Furthermore, we discuss the effect of storage temperature and cryoprotectants.

As the result, there were some structural changes such as vacuolar degeneration in the

cultured mucosal sheets using dimethyl sulfoxide (DMSO) as a cryoprotectant. Such changes were clearly observed at −80°C than at −196°C with DMSO. However, little morphological change was observed in both epithelial sheets cultured with glycerin. The cell viability analysed by flow cytometry showed that more than 62% of the cells kept their viability after freezing storage. These results suggest that the optimum condition of freezing for cultured epithelium were −196°C storage by slow cooling methods with glycerin as a cryoprotectant.

QUANTITATION OF THE SHAP-HA COMPLEX IN SERA FROM OA AND RA PATIENTS

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It was previously showed that SHAPs (serum-derived hyaluronan associated proteins) are firmly bound to hyaluronan (HA) in cultured fibroblasts, and found that SHAPs correspond to the heavy chains of inter-alpha-trypsin inhibitor (ITI) in serum and are covalently linked to HA by ester bond. Recently high amount of the SHAP-HA complex was detected in synovial fluid from human arthritis patients.

In this study, I deviced a simple detection methods by ELISA of the SHAP-HA complex and HA itself in serum and examined if there are differences between osteoarthritis (OA) and rheumatoid arthritis (RA) patients. I investigated sera from 130 patients (30 with OA and 100 with RA) and 100 normal controls. The results demonstrated that HA level increases about 1.5 times in all RA patients, but does not significantly change in OA patients. In some samples from RA patients, SHAP-HA complex level was 3-5-folds as much as normals, but in all OA samples the rather low level was observed compaired to normals.

EFFECTS OF SODIUM HYALURONATE ON AN EXPERIMENTAL OSTEOARTHRITIS IN RABBIT KNEES

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The aim of this study was to examine the effects of intraarticular administration of hyaluronan on cartilage degradation. Using a partial menisectomy model of osteoarthritis in the rabbit knee, the authors investigated the catabolic and anabolic changes induced by intraarticular injection of hyaluronan. To analyze anabolic changes, the authors measured cell proliferation by (^{3}H)thymidine, and proteoglycan biosynthesis by (^{35}S)sulfate and incorporation. For catabolic changes, messenger ribonucleic acid (mRNA) expression of interstitial collagenase (MMP-1), stromelysin 1 (MMP-3), and tissue inhibitor of metalloproteinase 1 (TIMP-1) in cartilage and

synovium were detected with reverse transcriptase polymerase chain reaction. A novel result for blocking development of early osteoarthritis in chondrocytes that total proteoglycan synthesis in hyaluronan treatment group was significantly higher than controls. At the messenger ribonucleic acid level in cartilage and synovium, hyaluronan seemed to inhibit MMP-3, but did not markedly affect MMP-1, and had a low detected frequency of detection of TIMP-1 production that similar to MMP-3 detection in hyaluronan treatment group. Thus hyaluronan affects cartilage both catabolism and anabolism to prevent the progress of osteoarthritis.

NEEDLESTICK INJURIES AMONG HEALTH CARE WORKERS: INTRODUCTION AND ASSESSMENT OF PREVENTIVE METHODS

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In recent years, occupational infections from needlestick injuries (NSIs) or sharp instrument injuries among health care workers have become a matter of concern. As the number of patients suffering from hepatitis C, acquired immune deficiency syndrome (AIDS) or pathogen-unknown bloodborne diseases increased, we should manage to control the problems. The purpose of this study was to assess the effectiveness of preventive methods for NSIs and sharp instrument injuries in a city hospital. In 1993, NSI Prevention Committee was organized, EPINet™ (Exposure Prevention Information Network) was adopted as a reporting form, and in 1994 four kinds of new safety devices were introduced for trial. The number of NSIs reported in this hospital for four years, from one year before the trial to two years after it, was analyzed. The results were as follows: 1) a total of 159 NSIs was reported for four years. The reported number of NSIs increased to about three times at the trial period (from 22 to 65 injuries), but decreased to 25 two years after the trial; 2) the NSIs which occurred on and after recapping needles were reduced; 3) the proportion of NSIs occurring while handling patients without infection of hepatitis C virus (HCV) increased; 4) the number of NSIs occurring while handling patients with HCV infection considerably decreased; 5) during the same period, the reported number of NSIs did not decrease in other four city hospitals given no any preventive methods. The present results indicate the importance of surveillance system using the NSIs reporting form and the effectiveness of safety devices to prevent NSIs.

HIGH PREVALENCE OF *PLASMODIUM MALARIAE* AND *P.OVALE* IN MALARIA PATIENTS ALONG THE THAI-MYANMAR BORDER, AS REVEALED BY ACRIDINE ORANGE STAINING AND PCR-BASED DIAGNOSES

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The prevalence of the four human malaria parasites was investigated among malaria patients at northern, central and southern towns in Thailand along the border with Myanmar between September 1995 and May 1996. Thin and thick smears were obtained from 548 Thai and Burmese patients by on site microscopy. Thin smears were examined by an acridine orange (AO) staining method, and many mixed infections with two to four species, including *P.malariae* and *P.ovale*, were detected. Parasite DNA was isolated from thick smears of all samples, and examined by two PCR-based diagnostic methods, microtiter plate hybridization (MPH) and a nested PCR method, both of which targets the same species-specific regions in the 18S rRNA genes. In both PCR diagnostic methods, many *P.malariae* and *P.ovale* infections were detected. The detection sensitivity of *P.malariae* infection was higher in the nested PCR method than the MPH. A total prevalence of *P.malariae* infection in this area reached 24.28% (133/548). In 16 of them, the size of nested PCR products amplified by the *P.malariae*-specific primer was about 20 bp shorter than the expected size of 115 bp. Moreover, two different bands, normal and shorter sizes, were detected by the nested PCR in four samples, suggesting that *P.malariae* isolates may be separated into two types, and that those with shorter products may be new variant form(s) with a nucleotide deletion in the target region. On the other hand, a total of 21 *P.ovale* infections (3.8%) were detected by the two PCR methods, and four of them were MPH-negative because of the same sequence variation at the probe region with that found previously in Vietnam. These results indicate that the prevalence of *P.malariae* and *P.ovale* along the Thai-Myanmar border is substantially higher than previously reported, with major implications for malaria treatment and control in this area.

VALUE OF ULTRASONOGRAPHY AND GALACTOGRAPHY IN DILATED DUCTS OR CYSTS OF THE BREAST

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Abnormal nipple discharge is a common clinical sign of breast disease, and it is important to know the presence or absence of intraductal tumor for treatment and planning follow-up examinations. Ultrasonography and galactography were performed in 40 patients with abnormal nipple discharge. We describe the usefulness of ultrasonography in the diagnosis of tumors in

dilated ducts or cysts compared with galactography. Intraductal tumors depicted by ultrasonography were demonstrated on galactogram as filling defects or ductal obstruction. In 16 of 20 (80%) cases with histopathological evidence of intraductal tumor, ultrasonography could depict the lesions as accurately as galactography.

THE CONTROL OF UPRIGHT STANDING POSTURE DURING LOW FREQUENCY LINEAR OSCILLATION

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We examined the effects of antero-posterior movement of sled on the human upright standing. Six healthy men were asked to stand on the platform in a linear accelerator (sled) in the dark. The sinusoidal acceleration intensity was graded form 0.02G to 0.04G, 0.06G and 0.08G, and the stroke length was from 6m to 10m and 14m. For the relation of acceleration to the induced body movement, the most marked change was found in the ankle. However, the head movement was distinctive, in that under 0.06G the head swayed with acceleration, but at 0.08G it was hold at a definite angle. The electromyographic discharge of the lower leg muscles showed co-activation of the gastrocnemius and tibialis anterior at intensity under 0.04G, while antagonistic response was found above 0.06G. During head movement, the neck muscles were slightly activated tonically at intensity under 0.06G, but they were markedly and tonically activated at 0.08G. We speculate that the sled oscillation caused the body sway in proportion to the acceleration, with the ankle joint playing a principal role. The neck movement also revealed the fixed position of the head in vertical position at intensity of 0.08G, indicating that the vestibulo-collic reflex might tonically active the neck muscles.

USE OF AN ARTIFICIAL NEURAL NETWORK FOR ANALYZING THE ECG WITH QS COMPLEX IN V1-2 LEADS

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The feed-forward neural network with the back-propagation algorithm is used to distinguish anterior wall myocardial infarction (AI) and Non-AI based on analyzing ECG. Data used in this study are 165 ECG records. They were all diagnosed AI by the commercially available computer-assisted ECG interpretation system (FCP4301), but only 80 of 165 cases were proved to suffer from AI by the history, physical examination, echocardiogram and other laboratory data, while the other 85 were not. In Non-AI cases, there are an abnormal Q-wave or small R progression in leads V_1 and V_2. The neural network was trained with the data from 40 cases of AI

and 42 cases of Non-AI, and then the performance of the neural network was tested with the remaining 83 patients (40 AI, 43 Non-AI) which had not been exposed to the network. The network correctly diagnosed 38 of the 40 cases with AI and 40 of the 43 cases without AI. The sensitivity and the specificity were 95% and 93%, respectively. The artificial neural network has the potential to improve the commercially available computer-assisted ECG interpretation system.

A COMPARISON OF SYMPATHETIC VASOMOTOR ACTIVITY AND CARDIOVASCULAR RESPONSES TO HEAD-UP TILT AND TO HEAD-UP SUSPENSION IN HUMANS

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The purpose of the present study was to test our hypothesis that the efferent muscle sympathetic nerve activity (MSNA) and cardiovascular responses to orthostatic stress are partly regulated by the activity of antigravity muscles. To test our hypothesis, we applied two different types of orthostatic stress for 3 consecutive, sequential 5-min periods at the levels of 20, 40 and 60° in 13 healthy male adults. First, the conventional head-up tilt (HUT) was used to provide an orthostasis with contraction of the antigravity muscles and produced by tilting the bed with the footboard positioned so that the subject stood on it. Second, head-up suspension (HUS) was used to minimize antigravity muscle activity during orthostasis and produced by a modified body harness. The shoulder straps of the harness were connected with the head end of the bed and then the bed was tilted after removing the footboard to cause the subject to be suspended without any contact of his feet with the footboard or the floor. We carried out intraneural recording of MSNA from the tibial nerve at the popliteal fossa by microneurographic technique. The heart rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and calf blood flow (CBF) were measured by ECG, autosphygmomanometer and impedance plethysmography respectively. The resting MSNA burst rate and burst incidence and the cardiovascular variables were similar during the HUT and HUS. Both techniques of orthostatic stress induced similar reflex responses at the low level (20°) of orthostasis. However, HUT at high levels (40 and 60°) induced significantly higher increase in the MSNA and HR than the HUS without changes in the arterial BP. The SV and CO were reduced more with a less reduction in the CBF during HUT at higher level (60°). In conclusion, the results provide evidence that the antigravity muscle activity along with the baroreflexes regulate the MSNA and cardiovascular responses during orthostatic stress by HUT in humans.