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FOR

GRADUATE STUDENTS

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Abstracts

ASSIGNMENT OF DISULFIDE BRIDGES IN THE FUSION GLYCOPROTEIN OF SENDAI VIRUS

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The mature fusion (F) glycoprotein of the paramyxovirus consists of two disulfide-linked subunits, the N-terminal F_2 and the C-terminal F_1 subunits, and contains 10 cysteine residues which are highly conserved at specific positions. The high level of conservation strongly suggests that they are indeed disulfide linked and play important roles in the folding and functioning of the molecule. However, it has not even been clarified which cysteine residues link the F_2 and F_1 subunits. We attempted to assign disulfide bridges in the purified Sendai virus F glycoprotein by fragmentation of the polypeptide and isolation of cystine-containing peptide and determination of their N-terminal sequences. The data demonstrate that all of the 10 cysteine residues participate in the disulfide bridges and that Cys-70, the only cysteine in F_2, and Cys-199, the most upstream cysteine in F_1, form the interchain bond. Of the remaining eight cysteine residues clustered near the transmembrane domain of F_1, the specific bridges identified are Cys-338 to Cys-347 and Cys-362 to Cys-370. Although no exact pairings between the subsequent four residues were defined, it seems likely that the most downstream, Cys-424, linked to Cys-394, Cys-399, or Cys-401. Thus we concluded that the cysteine-rich domain indeed contributes to the formation of a bunched structure containing at least two tandem cystine loops.

CHARACTERIZATION OF THE IMMUNOREGULATORY ACTION OF SAIKOSAPONIN-d

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The immunoregulatory action of saikosaponin-d (SSd), which was isolated from the root of *Bupleurum falcatum* L. and has a steroid-like structure, was examined on splenic T lymphocytes of C57BL/6 mice. SSd displayed a definite action *in vitro* to bidirectionally control the growth response of T lymphocytes stimulated by concanavalin A, anti-CD3 monoclonal antibody or calcium ionophore A23187 plus phorbol 12-myristate 13-acetate. Low concentrations (1-3 μg/ml) of SSd up-regulated the responses to suboptimum stimuli of agonists, particularly during the relatively late stage of the responses, whereas it down-regulated the responses to supraoptimal stimuli. Under appropriate experimental conditions, SSd promoted interleukin-2 (IL-2) production and IL-2 receptor expression. It also accelerated c-*fos* gene transcription, but it did not modulate the level of tyrosine phosphorylation of cellular proteins. We concluded from these results that SSd uniquely modulates T lymphocyte function, and that at least one target of the action of SSd is located at or before the step of c-*fos* gene transcription and after T-cell receptor/CD3-mediated protein tyrosine kinase activation.

EVIDENCE OF MULTISTAGE CROSSTALK BETWEEN CYSTEINE-BASED AND PHOSPHORYLATION-DEPENDENT SIGNAL PATHWAYS ACTIVATING $p60^{c\text{-}src}$

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It is established that non-receptor tyrosine kinases are subjected to regulation by protein phosphorylation/dephosphorylation when a receptor ligand interaction takes place. We found that the cysteine thiol-group reactive heavy metal mercury ($HgCl_2$) activated non-receptor tyrosine kinase $p60^{c\text{-}src}$ in NIH3T3 cells. This mercury action seemed to be specific for cysteine residues as it was neutralized by thiol-donating chemicals. We here defined two novel cysteine-based pathways upregulating $p60^{c\text{-}src}$. One of them was through binding of mercury to non-$p60^{c\text{-}src}$ proteins on cell surface. This induced secondary aggregation of intracellular $p60^{c\text{-}src}$ and selected proteins which should break the phosphorylation-dependent regulation by conformational change. In addition, this pathway in part mobilized tyrosine phosphatase activity to upregulate $p60^{c\text{-}src}$. The other pathway was through direct binding of mercury to the cysteine thiol-group(s) of $p60^{c\text{-}src}$ in subcellular forms. The mercury-induced direct modification of conformation of $p60^{c\text{-}src}$ should antagonize the phosphorylation-dependent regulation. These results suggest multiple stage linkage between cysteine-based (redox-linked) and phosphorylation-dependent regulatory mechanisms focusing $p60^{c\text{-}src}$.

THERMAL DEPENDENCY OF SKIN SYMPATHETIC NERVE ACTIVITY TO THE GLABROUS AND HAIRLY SKIN IN HUMANS

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The sympathetic outflows from the central nervous system are distributed to different effector organs according to regional differentiation. The ambient temperature is known to alter skin sympathetic nerve activity (SSNA), and modulate thermal sweating and vasoconstriction in the glabrous and hairy skin. To investigate the effects of ambient temperature on the sudomotor and vasoconstrictor components of sympathetic outflow, SSNA was simultaneously measured by microneurography from post-ganglionic nerve fibers in the tibial and the peroneal nerves at the popliteal fossa (double recording). When the ambient temperature was raised from 25°C to 34°C, both sudomotor and vasoconstrictor components of SSNA were enhanced in the peroneal nerve, but were suppressed in the tibial nerve. The sudomotor and vasoconstrictor sympathetic outflows were elevated in both nerves when the temperature was lowered from 34°C to 18°C. Our results suggest that the sudomotor and the vasoconstrictor components of SSNA are differently modulated by ambient temperature. The difference in sudomotor and vasoconstrictor

components of SSNA in the tibial and the peroneal nerves at various ambient temperature may be responsible for the differences observed in sweating and vasoconstriction in the glabrous and hairy skin.

MEMORY IMPAIRMENT AND NEURONAL DYSFUNCTION AFTER CONTINUOUS INFUSION OF ANTI-NERVE GROWTH FACTOR ANTIBODY INTO THE SEPTUM IN ADULT RATS

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Nerve growth factor (NGF) plays an important role in the survival and maintenance of cholinergic neurons in the central neuronal system. In senile dementia of Alzheimer type (SDAT), learning and memory are impaired by the loss of neurons in the magnocellular cholinergic neuronal system. Therefore, it is of interest to investigate the role of NGF in SDAT. We have reported that the specific Fab' fragment of anti-NGF antibody impairs learning and memory and alters nuclear morphology in the hippocampus and parietal cortex. In this study, we investigated whether memory impairment and neuronal dysfunction were produced by continuous infusion of anti-NGF antibody into the right septum in adult rats. Step-through latency of anti-NGF antibody-treated group was shorter than that of control group. The activities of choline acetyltransferase and cholinesterase significantly decreased in the right hippocampus immediately after 2 weeks-continuous infusion. However, 2 weeks after the end of continuous infusion both behavioral and biochemical parameters recovered from dysfunction. These results suggest that lack of NGF may be one of the cause of SDAT.

EFFECT OF INTERLEUKIN-2 ON THE PRODUCTION OF PROGESTERONE AND PGE_2 IN HUMAN FETAL MEMBRANES AND ITS CONSEQUENCES FOR PRETERM UTERINE CONTRACTIONS

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Our objective was to clarify the mechanism of uterine contraction induced in pregnant women by intrauterine bacterial infection. The concentration of interleukin-2 (IL-2) was measured in amniotic fluids which had been obtained by amniocentesis, transvaginal amniotomy or by transuterine amniocentesis performed at caesarean section from 50 pregnant women. The concentration of IL-2 of the cases with intrauterine infection was significantly higher than that of those without intrauterine infection at preterm. The same tendency was found at fullterm.

Scatchard analysis demonstrated the presence of an IL-2 receptor in the fetal membranes. We collected the fetal membranes aseptically for the measurement of progesterone and prostaglandin E_2 (PGE_2) by radioimmuno assay following incubation with various concentrations of IL-1 and IL-2 at 37°C for 16 h. The production of progesterone was significantly inhibited by 10 pM IL-2, but not by 10 pM IL-1. The production of PGE_2 was significantly accelerated by either IL-1 or IL-2 at a dose of 10 pM. The inhibitory effect of IL-2 on the production of progesterone was unaffected by indomethacin, which inhibits the production of arachidonate cyclooxygenase metabolites such as PGE_2. Our present data suggest that the presence of intrauterine bacterial infection may stimulate the intrauterine production of IL-2, and that the reduction of progesterone resulting from the stimulation of IL-2 may in part explain the mechanism of uterine contraction associated with intrauterine infection during pregnancy.

EFFECT OF NEUROPEPTIDE Y ON LUNG VASCULAR PERMEABILITY AND ITS RECEPTOR SUBTYPE — WITH PARTICULAR REFERENCE TO NEUROGENIC PULMONARY EDEMA

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The effects of neuropeptide Y on the lung vascular permeability in terms of the perivascular carbon deposits were examined by in-vitro study, using isolated lung perfusion preparations from rats. The number of extravasated carbon particle deposits subsequent to tracheal application of neuropeptide Y during spontaneous respiration increased in a dose-dependent manner. And in the presence of β-blocking agents, NE augmented the effect of NPY to increase the perivascular carbon deposits.

Although the effects of peptide YY and $NPY_{18\text{-}36}$, an antagonist of NPY, were also examined to determine the receptor subtype which corresponded to the effect of NPY, the potency of PYY was much smaller than that of NPY, and $NPY_{18\text{-}36}$ significantly decreased the NPY-induced increase in perivascular carbon deposits. So, we may conclude that NPY increases vascular permeability in the pulmonary circulation, acting not on Y_1, Y_2, but on Y_3-like receptors.

Furthermore, the role of NPY in the development of neurogenic pulmonary edema, e.g., fibrin induced pulmonary edema of rats, was examined by in-vivo study. Pretreatment of $NPY_{18\text{-}36}$ decreased the incidence of pulmonary edema from 100% to 30%. From these results, we conclude that the effect of NPY on increasing lung vascular permeability may play an important role in the development of neurogenic pulmonary edema.

THE EFFECT OF NITRIC OXIDE ON CHONDROCYTES AND LYMPHOCYTES

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Nitric oxide has been shown to play important roles in the regulation of immune function, blood vessel dilation, brain and peripheral nervous systems and interactions of numerous other cells and tissues.

It has been shown that chondrocytes produce nitric oxide in response to cytokines and/or LPS, and nitric oxide synthesis is increased in synovial fluid and serum in rheumatic diseases. There is an abundance of activated lymphocytes in synovium and synovial fluid with inflammation such as pyogenic or rheumatoid arthritis. To investigate the interaction between chondrocytes and lymphocytes, we measured nitric oxide in the culture medium of chondrocytes and/or lymphocytes, and examined the effects of nitric oxide on their proliferation.

Concanavalin-A (ConA)-activated lymphocytes markedly induced the production of nitric oxide from chondrocytes. The nitric oxide significantly inhibited the proliferation of both the chondrocytes themselves and the lymphocytes. When culture supernatant from ConA-activated lymphocytes was added to culture of chondrocytes, a large amount of nitric oxide was produced. N^G-monomethyl-L-arginine (MMA) significantly eliminated both the production of nitric oxide and the inhibition of chondrocyte and lymphocyte proliferation. Furthermore, when MMA was added to the mixed culture of chondrocytes and lymphocytes with ConA, their proliferation was markedly enhanced compared with individual summation. These results indicate that chondrocytes produce not only nitric oxide but also a lymphocyte stimulating factor, the effects of which are, however, usually masked by the nitric oxide.

THE ROLE OF TGF-β1 IN INDUCTION, PROLIFERATION, AND PHENOTYPIC EXPRESSION DURING CHONDROGENESIS

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The function of TGF-β1 during chondrogenesis was investigated in various tissues in organ culture. Periosteum, muscle, fascia lata, patellar tendon, Achilles tendon, joint capsule, infrapatellar fat pad, meniscus, and skin explants were harvested from 2 month old New Zealand white rabbits. [^{3}H]-thymidine and [^{35}S]-sulfate incorporations were monitored at various time intervals. Cartilage formation was evaluated by histomorphometry and quantitative collagen typing. Chondrogenesis was induced by TGF-β1 in all tissues except skin, however only periosteum formed cartilage in the absence of TGF-β1. The chondrogenic inducing effect was dose-dependent with each tissue type responding differently. Cell proliferation was stimulated by TGF-β1 in the first 2 days of culture with the periosteal explants, but was significantly reduced on day

10. Proteoglycan synthesis was increased 30-to 40-fold in the periosteal explants treated with TGF-β1. Cartilage formation as assessed by histomorphometry, and type II collagen production were also dramatically increased by TGF-β1. Chondrogenic stimulation was maximal following 2 days of exposure to TGF-β1, and not further enhanced by 14 days treatment with TGF-β1. These results suggest that the chondrogenic stimulating effect of TGF-β1 occurs mainly at the stage of chondrogenic induction.

EFFECTS OF INSULIN-LIKE GROWTH FACTOR I AND GLUCOSE ON CULTURED BOVINE RETINAL PERICYTES

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Retinal pericytes are affected in the early stages of diabetic retinopathy, but the mechanism is unclear. Recently studies investigating the relationship between insulin-like growth factor I (IGF-I) and diabetic retinopathy have yielded conflicting results. To explore the possible contribution of IGF-I to the development of diabetic retinopathy, we examined the effect of IGF-I on retinal pericytes. IGF-I significantly increased [^{3}H]-thymidine incorporation into DNA, and its effect was completely inhibited by IGF-I receptor antibody. At a normal glucose concentration (5.5 mM), [^{3}H]-thymidine incorporation increased and reached a plateau. At a 40 mM glucose concentration, however, [^{3}H]-thymidine incorporation increased significantly in a significantly in an IGF-I-dependent manner within the range tested. Expression of IGF-I receptors on intact cells differed according to the glucose concentration. Specific binding of IGF-I on the pericytes was increased significantly with 40 mM glucose, compared with 5.5 mM glucose, and initial binding at 40 mM glucose was about twice that at 5.5 mM glucose. These results suggest that retinal pericytes may be regulated by IGF-I via IGF-I receptors and that the number of these receptors may be increased by hyperglycemia. Thus, IGF-I may play an important role in the development of diabetic retinopathy.

INCREASE IN Pit-1 mRNA IS NOT REQUIRED FOR THE ESTROGEN-INDUCED EXPRESSION OF PROLACTIN GENE AND LACTOROPH PROLIFERATION

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Estrogen has been shown to stimulate lactotroph proliferation and expression of the prolactin (PRL) gene. Recently it has been established that Pit-1, a pituitary-specific transcription factor,

is required for lactotroph proliferation. Furthermore, *in vitro* studies showed that an increase in the PRL promoter activity caused by estrogen was dependent on the amount of cotransfected Pit-1-expressing plasmid. These findings led us to examine whether the induction of Pit-1 mRNA is required for the estrogen-increased PRL gene expression in the rat anterior pituitary *in vivo*. Short-term estrogen treatment was achieved by means of a single intramuscular injection of estradiol dipropionate. DNA synthesis, the levels of PRL and Pit-1 messenger RNAs in the anterior pituitary, were determined. Estradiol dipropionate resulted in a significant increase in DNA synthesis 24 hours after administration and in PRL mRNA after 48 hours. In contrast, the Pit-1 mRNA level was not altered. Since Pit-1 is expressed not only in lactotrophs but also in somatotrophs and thyrotrophs, and the lactotroph cell population has been reported to be less than 10% in the pituitary, the change in the Pit-1 mRNA level in lactotrophs was not seen following only short-term estrogen treatment. An increase in the lactotroph cell population was therefore achieved by chronic estrogen treatment (subcutaneous implantation of a silastic tube containing 17β-estradiol powder for 30 days). This treatment resulted in the marked proliferation of lactotrophs and a 3-fold increase in PRL mRNA. However, no alteration in Pit-1 mRNA was observed. These results suggest that the increase in Pit-1 mRNA is not required for the estrogen-induced lactotroph proliferation or PRL gene expression.

PROGNOSTIC SIGNIFICANCE OF p53 MUTATIONS AND 3p DELETIONS IN PRIMARY, RESECTED NON-SMALL CELL LUNG CANCER

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Among the genetic abnormalities identified in non-small cell lung cancer (NSCLC) thus far, mutations in the p53 gene on chromosome 17p and an allelic deletion of chromosome 3p appear to be the most frequent targets. A frequent occurrence of 3p deletion was also observed in small cell lung cancer, strongly suggesting the presence of a tumor suppressor gene(s) for lung cancer in this chromosomal region. We evaluated the prognostic significance of p53 mutations and an allelic loss of chromosome 3p in 71 patients with NSCLC who underwent potentially curative resection. p53 mutations were detected in 35 cases (49%) and 3p deletions were observed in 34 of 70 informative cases (49%). The presence of the p53 mutation was associated with a shortened survival in all patients ($p=0.014$ by log-rank test), including those in early stages of the disease (stage I or II, $n=48$) ($p=0.016$ by log-rank test). Multivariate analysis by the Cox proportional hazards model also revealed that p53 mutation was an independent yet unfavorable prognostic factor ($p=0.013$). Patients with 3p deletion tended to have a poorer prognosis, but not to a statistically significant extent. NSCLC patients with p53 mutations may be defined as candidates for new investigational therapeutic approaches.

CEREBROSPINAL FLUID LEVELS OF SUPEROXIDE DISMUTASES IN NEUROLOGICAL DISEASES DETECTED BY SENSITIVE ENZYME IMMUNOASSAYS

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We measured cerebrospinal fluid (CSF) levels of Cu/Zn superoxide dismutase (Cu/Zn SOD) and Mn superoxide dismutase (Mn SOD) using enzyme immunoassays in 196 neurological patients and 44 controls. The mean Cu/Zn SOD level was 55.8 ± 27.6 (SD) ng/ml and the Mn SOD, 8.0 ± 2.5 ng/ml in the controls. Cu/Zn SOD or Mn SOD levels showed neither age- nor sex-related differences in the controls. Both SODs were markedly elevated in cerebrovascular diseases, bacterial mentingitis and encephalitis. Mn SOD alone was significantly elevated in neurodegenerative diseases. We compared SODs with CSF levels of neuron-specific enolase (NSE) and S-100b protein (S-100b) in cerebral infarction and bacterial meningitis. Both SODs were correlated with NSE and S-100b in patients with cerebral infarction, but not in those with bacterial meningitis. This means that elevations of SODs in CSF may not only be due to leakage from damaged nervous tissues, but also to the induction of SOD in lesions. We conclude that the mean SOD levels were elevated in various neurological diseases, and their varied magnitudes may be associated with the underlying diseases.

INTERNAL BILIARY DRAINAGE, UNLIKE EXTERNAL DRAINAGE, DOES NOT SUPPRESS THE REGENERATION OF CHOLESTATIC RAT LIVER AFTER PARTIAL HEPATECTOMY

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We have previously shown that hepatic cells proliferate by obstructive jaundice alone without partial hepatectomy, and found that the external biliary drainage for obstructive jaundice markedly suppressed liver regeneration after partial hepatectomy. In the present study, we produced an experimental model for internal biliary drainage, in which bile was drained into the stomach of rat fed with liquid diet. The regeneration capacity of the liver was assessed by the induction of DNA polymerase α activity as well as by the mitotic index of hepatocytes. A remarkable difference was observed in the regeneration capacities of cholestatic livers between two groups wherein jaundice was released by either the internal or the external biliary drainage prior to hepatectomy. After 5 days of internal biliary drainage, the regeneration capacity remained at a level comparable to those of sham-operated control rats, in sharp contrast to the impaired regeneration after the external biliary drainage. These results clearly indicate that internal biliary drainage is preferable for releasing the biliary obstruction before partial resection of the cholestatic liver.

EXPRESSION OF PLASMINOGEN ACTIVATORS AND THEIR INHIBITORS IN HUMAN PANCREATIC CARCINOMA: IMMUNOHISTOCHEMICAL STUDY

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Expression of plasminogen activators (PA), tissue type (t-PA) and urokinase type (u-PA), as well as PA inhibitors (PAI), type-1 (PAI-1) and type-2 (PAI-2), were investigated immunohistochemically in 97 human pancreatic carcinomas. u-PA expression predominated in pancreatic carcinomas when compared with t-PA, u-PA expression in 76 specimens (78.4%) and t-PA in eight specimens (8.2%). PAI-1 expression was detected in 80 carcinoma specimens (82.5%) and PAI-2 in 79 carcinoma specimens (81.4%). PAI-2 expression was significantly lower in carcinomas with peritoneal metastasis ($p < 0.02$). Strong PAI-2 expression was associated with significantly higher survival than negative or weak PAI-2 expression ($p < 0.05$). We conclude that immunohistochemical analysis of PAI-2 expression in pancreatic carcinomas may yield important prognostic information.

DECREASE IN CYTOCHROME *c* OXIDASE AND CYTOCHROME OXIDASE SUBUNIT I MESSENGER RIBONUCLEIC ACID LEVELS IN PREECLAMPTIC PREGNANCIES

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Objective: The purpose of this study was to elucidate the possible relationship between mitochondrial gene expression and placental dysfunction.
Methods: We measured the activity of cytochrome *c* oxidase and the expression of cytochrome oxidase subunit I in mitochondria from placentae of women who were appropriate for gestational age (AGA) and those with preeclampsia. In addition, the amounts of normal mtDNA and deleted mitochondrial DNA were examined in the two groups by Southern blot analysis and polymerase chain reaction, respectively.
Results: Cytochrome *c* oxidase activity and expression of cytochrome oxidase subunit I were significantly lower in the preeclamptic group than in the AGA group. There were no differences in the amounts of mitochondrial DNA between the two groups. In addition, no mutant mitochondrial DNA with a 4,977 base pair deletion was detected in the two groups.
Conclusion: These results suggest that reduced expression of the mitochondrial gene is involved in placental dysfunction in preeclamptic pregnancy.

MODULATION OF SINUS RATE BY ATRIAL CELL COUPLING

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We examined the electrotonic interactions between the sinoatrial (SA) node and atrial muscle. Isolated rabbit SA node cells were coupled to a membrane model of a single atrial cell via an external circuit which mimics the gap junctional connection between cardiac cells. Step increases of the coupling conductance (Gc) induced a progressive prolongation of spontaneous cycle length (SCL) associated with characteristic changes in action potential configuration. At Gc above a certain level, the spontaneous activity became irregular and finally stopped. We defined the "threshold Gc" as the Gc at which the SCL irregularity (SD/mean) was greater than 0.3. The threshold Gc for a single SA node cell was 0.3 ± 0.04 nS (mean $\pm$ SEM, n=16). In the presence of acetylcholine (ACh 0.05—0.2 μM), the coupling-induced inhibition of spontaneous activity was enhanced and the threshold Gc was decreased in a concentration-dependent manner (0.14 ± 0.03 nS at 0.1 μM ACh). These findings suggest that the regular pacemaker activity of a single SA node cell is easily inhibited when it is coupled directly to an atrial cell and ACh enhances the coupling-induced inhibition of spontaneous activity.

DIFERENCE IN CHARACTERISTICS OF MUCOSAL CELLS AND EPIDERMAL CELLS AS MATERIALS FOR CULTURED EPITHELIAL GRAFTING

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The characteristics of cultured mucosal cells from the oral mucosa and epidermal cells from the skin were investigated. Total cell counts showed that mucosal cells possessed greater proliferating ability than epidermal cells. The results of MTT assay confirmed this observation and also suggested that the mucosal cells maintained biological activity longer than epidermal cells. The most important morphological characteristics of mucosal cells in culture were their low grade of differentiation. Interestingly, the epidermal cells showed enucleation and keratinization progressively during culture, while the mucosal cells showed no obvious enucleation when examined by light microscopy. Transmission electron microscopy showed smaller number of desmosomes in cultured mucosal cells than epidermal cells. The results of this study reveal a similarity in the process of fabricating mucosal and epidermal cell sheets, although the mucosal cells still maintained its original nature biologically.

MOLECULAR CLONING OF THE RAT THYROXINE-BINDING GLOBULIN GENE AND ANALYSIS OF ITS PROMOTER ACTIVITY

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We cloned the rat thyroxine-binding globulin (rTBG) gene, characterized its organization and studied its promoter activity and regulation. A genomic DNA library was constructed and screened using a rTBG complementary (c) DNA as a probe. A 8.6 kilobasepair (kbp) clone was partially sequenced and compared with the sequence of the previously cloned cDNA. It helped complete the cDNA sequence and identify the first noncoding exon (exon 0). The transcription start site (TSS) was identified using an RNase protection assay. The rTBG genomic clone contained 1.2 kbp 5′-flanking and 1.7 kbp 3′-flanking regions. The sizes of exons and introns of the rTBG gene are similar to those of the human TBG gene. The 5′-flanking region contains a TATA box, a CAAT box and a consensus sequence for hepatocyte nuclear factor 1 (HNF-1) binding site.

We tested the promoter activity of the 1.2 kbp 5′-flanking region using a luciferase reporter plasmid. When transfected into a hepatocyte-derived cell line (HepG2), the plasmid construct containing the fragment −1227 to +11 (TSS: +1) showed a 9-fold increase in luciferase activity compared with that of a promoterless luciferase vector. No promoter activity was detected in nonhepatocyte-derived cell line (COS1). Serial 5′-deletion revealed that the construct containing the fragment −53 to +11 showed no significant increase of luciferase activity. These results suggest that the region −180 to −53, containing HNF-1 binding site, is essential to the liver-specific expression.

We previously reported down-regulation of rTBG mRNA by T_3 *in vivo*. The present study failed to show T_3 effect on the promoter activity of the 1.2 kbp 5′-flanking region of the rTBG gene.

CHROMOSOMAL MAPPING OF GENETIC LOCUS ASSOCIATED WITH THYMUS ENLARGEMENT IN BUF/Mna RATS

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The thymoma-prone rat of the BUF/Mna strain is a useful model for human thymona. In this strain, thymoma development is regulated by a single autosomal susceptible gene, Tsr-1. At prethymoma age, BUF/Mna rats have extremely large thymuses, compared with those of other strains of rats. Genetic studies in crosses between BUF/Mna rats with large thymuses and WKY/NCrj rats with small thymuses suggested the presence of some autosomal genes, Ten, which contribute to thymus enlargement in a backcross population. We performed linkage studies between Ten and micro-satellite markers in backcross rats of (WKY/NCrj × BUF/Mna)

F1 × BUF/Mna. Myosin light chain, muscle 2 (MYL2) locus, which is assigned to rat chromosome 1, strongly cosegregated with thymus size in the backcross population. D13N2, Asp-, Gly-, Glu- and Leu-tRNAs cluster (TRAGGL), ATPase alpha-2 (ATPA2S) loci, which are assigned to rat chromosome 13, also strongly cosegregated with thymus size in the backcross population. We concluded that Ten-1 is located near MYL2 locus on chromosome 1, and Ten-2 is located between D13N2 and TRAGGL loci on chromosome 13. This result may provide an approach to clone Tsr-1, which could be allelic to either Ten-1 or Ten-2.

A PHENOTYPICALLY NEUTRAL DIMORPHISM OF PROTEIN S

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During the course of structural gene analysis of a family with hereditary protein S deficiency, we found a novel DNA polymorphism; A or G variation at nucleotide 732 in exon 6 of the protein S gene. This A-to-G transition would result in a substitution of Glu (*G*AG) for Lys 155 (*A*AG) in the second EGF domain of protein S. A genetical analysis in the protein S-deficient family revealed that the A-to-G exchange was present in normal family members having normal protein S activity as well as the affected individual. The same base substitution was found in two individuals from an unrelated kindred, who also had normal amounts of functional protein S levels. An Analysis of normal genomic DNA samples showed that the A-to-G transition is observed at a frequency of 0.82% in the Japanese population. These findings suggest that the A-to-G exchange, resulting in a substitution of Glu for Lys 155 in the second EGF domain, is not responsible for protein S deficiency, but a phenotypically neutral polymorphism, and that the second EGF domain of protein S would not play an important role in protein S function.

THE CLONING OF PIG-A, A GENE RESPONSIBLE FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia by the presence of abnormal subpopulations of blood cells that are deficient in surface expression of glycosylphosphatidylinositol (GPI)-anchored proteins. A complementary DNA encoding a human protein termed PIG-A (phosphatidylinositol glycan-class A) was obtained by expression cloning using a mutant cell line deficient in biosynthesis of GPI-anchor. PIG-A was necessary for synthesis of N-acetylglucosamine-phosphatidylinositol, the very early intermediate in GPI-anchor biosynthesis. The PIG-A gene of at least 17 kb long and six exons was also cloned and

mapped on the X chromosome, 22.1. The 5′ flanking region has no TATA-like sequence, but includes four CAAT boxes, two AP-2 sequences, and a CRE sequence, some of which are present in regions necessary for the promoter activity. PIG-A cDNA corrected the deficient phenotype of cell lines affected by PNH, and a somatic mutation that caused loss of function of PIG-A was identified in one patient, strongly suggesting that PIG-A is the gene responsible for PNH.

SOMATIC MUTATIONS OF PIG-A GENE FOUND IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematological disorder caused by a deficiency in the synthesis of the glycosyl-phosphatidylinositol molecules that anchor proteins to the cell membrane. The defect has been pinpointed to the step catalyzed by the α-1,6-N-acetylglucosaminyl-transferase. Recently, the gene termed PIG-A necessary for this step was cloned. In this study, we analyzed the PIG-A gene using granulocytes from 16 patients with PNH to know whether PIG-A is the gene responsible for all cases of PNH and to characterize further the somatically acquired mutations of this gene. The PIG-A mRNA isolated from granulocytes, reverse transcribed, amplified by PCR, and then analyzed by agarose gel electrophoresis or cloned into plasmids for the functional assay or nucleotide sequencing. Three patients had size abnormalities of PIG-A transcripts and the sequence analysis showed that these size abnormalities were formed by abnormal splicing due to the somatic mutation within splice site. In two patients very low level of the PIG-A transcripts were found. Eleven patients had transcripts of normal size, but the transfection assay revealed the presence of non-functional transcripts in each of them. The sequence analysis of non-functional clones in these patient demonstrated nonsense mutations in two patients, missense mutations in three patients and frame shift mutations in six patients. The present finding that all patients with PNH studied so far had the abnormality of the PIG-A gene, strongly suggests that PIG-A is the target gene for most, if not all, cases of PNH.

IDENTIFICATION OF THE PREGNANCY RESPONSIBLE FOR GESTATIONAL TROPHOBLASTIC DISEASE BY DNA ANALYSIS

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In three cases of choriocarcinoma, genetic loci including a variable number of tandem repeat

regions were amplified by the polymerase chain reaction method on DNA from three established cell lines and lymphocytes of patients and their husbands to identify the responsible pregnancy. Case 1 patient, from whom NaUCC-3 was derived, had only one full term fetal death. Case 2 patient, from whom NaUCC-4 was derived, had one normal delivery followed by one complete molar delivery and one normal delivery. Case 3 patient, from whom NaUCC-2 was derived, had one normal delivery followed by one complete molar delivery. In case 1, NaUCC-3 was found to be of parental origin and to have derived from the pregnancy with full term fetal death. In cases 2 and 3, NaUCC-4 and NaUCC-2 were of androgenetic origin and were found to have derived from the pregnancy with complete hydatidiform moles. We also conducted restriction fragment length polymorphism method using case 1 samples, and it confirmed the results based on the polymerase chain reaction method product patterns. All nine cases of hydatidiform moles were of androgenetic origin and three cases of invasive moles were of androgenetic origin. The utilization of the polymerase chain reaction method thus makes it possible to identify easily the responsible pregnancy for choriocarcinoma using only a few specimens without isotopes.

DETECTION OF IgE ANTIBODY AGAINST *CANDIDA ALBICANS* ENOLASE AND ITS CROSS-REACTIVITY TO *SACCHAROMYCES CEREVISIAE* ENOLASE

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Candida albicans 46 kDa protein, a glycolytic enolase enzyme, is an important allergen of the yeast. The purpose of the study was to detect circulating IgE and IgG antibodies against *C. albicans* enolase. We isolated 46 kDa *C. albicans* enolase using sequential DEAE Sephacel and P11 column chromatography from spheroplasts of *C. albicans*, and detected IgE and IgG antibody against *C. albicans* enolase (CAE) by immunoblotting. Cross-reactivity of enolse of *C. albicans* and *Saccharomyces cerevisiae* was also examined by immunoblotting and immunoblot inhibition test. Among 54 sera with positive IgE RAST to *C. albicans*, IgE antibody against CAE was detected in 20 sera (37%) and IgG antibody in 27 sera (50%). The allergenic potency of CAE was confirmed using a skin prick test in three patients. Simultaneous IgE binding to *S. cerevisiae* enolase was only observed in 4 out of 20 sera reacting to CAE. Results of IgE immunoblotting inhibition test demonstrated that pre-treatment of sera with CAE completely inhibited IgE antibody binding to *S. cerevisiae enolase*, whereas the latter only partially inhibited IgE antibody binding to CAE. These results suggest that CAE shares some cross-reacting epitopes with *S. cerevisiae* enolase, representing minor components of CAE but dominant segments of *S. cerevisiae* enolase. Detection of IgE antibody to CAE may indicate a specific sensitization to *C. albicans.*

ICAM-1 AND LFA-1 ARE INVOLVED IN PROTECTION MEDIATED BY $CD3^+TCR\alpha\beta^-$ T CELLS AT THE EARLY STAGE AFTER INFECTION WITH *LISTERIA MONOCYTOGENES* IN RATS

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To investigate the significance of intercellular adhesion molecule-1 (ICAM-1) and leukocyte function-associated antigen-1 (LFA-1) in host defense against infection with intracellular parasites, we examined the effects of *in vivo* pretreatment with monoclonal antibodies (mAbs) to ICAM-1 (1A29) and LFA-1α (WT-1) on the protection against infection with *Listeria monocytogenes* in Fisher F344/N rats. Expressions of ICAM-1 and LFA-1α molecules on T cells in spleen, liver and peritoneal cavity of rats were down-regulated after intraperitoneal administration with daily doses of 300 μg of either 1A29 or WT-1 for 10 days. The survival rate of rats inoculated with viable *Listeria* was significantly reduced by *in vivo* pretreatment with 1A29 together with WT-1 for 10 days but not by *in vivo* pretreatment with control mAb. The numbers of bacteria in the spleen in rats pretreated with both 1A29 and WT-1 were significantly increased on day 3 and day 6 after infection with 1×10^7 of viable *Listeria* corresponding to 1/30 of LD_{50} to normal rats. Thus, the resistance against listerial infection was severely impaired by the combinational pretreatment with mAbs to ICAM-1 and LFA-1α. As shown in our previous report, the early appearance of $CD3^+TCR\alpha\beta^-$ T cells, presumably $TCR\gamma\delta$ T cells, was evident in the peritoneal cavity and liver of control rats at the early stage after listerial infection, while this was suppressed at this stage in rats pretreated with both 1A29 and WT-1. These results suggest that ICAM-1 and LFA-1 adhesion pathway may be critically involved in protective roles of $CD3^+TCR\alpha\beta^-$ T cells at the early stage in rat listeriosis.

EVALUATION OF THE PERFORMANCE OF WHOLE-BODY POSITRON IMAGING SYSTEM WITH ATTENUATION CORRECTION

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The PET scanner with ring detectors has been modified to accomplish whole-body imaging with attenuation correction. To evaluate the performance of this whole-body positron imaging system with attenuation correction, phantom studies and clinical studies in seven patients were performed. The resolution (FWHM) at the center of the field was 8.6 mm. The counts on the images with attenuation correction were linearly related to accumulation of the radiopharmaceuticals, and attenuation correction was made properly. The one minute acquisition time of the transmission scan per one bed position and the 30 seconds acquisition of the emission scan provided us sufficient quality of the images in both resolution and contrast. The transmission scan

from the top of the head to the thigh, about 110 cm in length, required about 36 minutes, and one emission scan required about 18 minutes. The total study time, for one transmission scan and three sequential emission scans, was about 1.9 hours and the total scaning time was 1.5 hours. In clinical studies, attenuation correction made discrimination of organs more clearly. It would facilitate to detect tumors, especially the tumors in the high attenuation organs or matters. Using this system made us possible to get the quantitative whole body images with adequate diagnostic quality within a reasonable scanning time.

AN ULTRASTRUCTURAL STUDY OF EXTRACELLULAR FIBRILLAR COMPONENTS OF DEVELOPING MOUSE MANDIBULAR CONDYLE WITH SPECIAL REFERENCE TO TYPE VI COLLAGEN

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The localization of type VI collagen in the mouse mandibular condyle was examined from newborn to 8 weeks of age. Immunohistochemical staining with anti-type VI collagen antibody was strongly positive in the hypertrophic zone and moderately positive in the fibrous zone and the outer periphery of the proliferative zone, but negative in the inner area of the proliferative zone and mature zone. After ATP treatment, type VI collagen periodic fibrils with about 80 nm intervals were frequently observed only in the fibrous zone. They occurred mainly in the superficial area of this zone, where striated collagen fibrils were sparse, while a few were noted in the inner area, where bundles of collagen fibrils were abundant. From these distributional differences of both components, a sub-zonation of fibrous zone into superficial and inner areas was suggested. Moreover, with ATP treatment there were fewer type VI collagen periodic fibrils formed with increasing age (8 weeks). Testicular hyaluronidase digestion before ATP treatment facilitated the formation of periodic fibrils, in all the ages examined, in the intercellular space and around the fibroblastic cells. The interaction of type VI collagen with other components such as collagen fibrils, glycosaminoglycans or proteoglycans may play a role in maintaining the structural integrity of extracellular matrix in the mouse mandibular condyle.

EVOKED OTOACOUSTIC EMISSIONS IN COURSE OF SUDDEN DEAFNESS

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To identify damaged region which is responsible for sudden deafness (SD), minimum

detectable levels of evoked otoacoustic emissions (EOAEs) and pure tone hearing levels were recorded. The measurement were archived once a week (3 times or more in total) in SD cases until hearing level had fixed and also in normal hearing subjects. 1 kHz short tone burst was fed into an acoustical probe and the output of microphone was averaged with 100 times. SD subjects were classified into four (complete, prominent, partial recovery and unchanged) groups according to the degree of hearing improvement.

EOAE detectable levels remained unchanged in the normal hearing subjects. Using a linear regression analysis, the complete recovery group had a positive correlation between the hearing levels and EOAE detectable levels. Some degree of correlation was found in the prominent and partial recovery groups while no correlation in the unchanged group. Some cases showed discrepancies in the relation between them.

These results can be explained by the hypothesis that there are multiple sites of damage in sudden deafness. The damaged region may be the hair cell, stria vascularis, Reissner's membrane and/or spiral ganglion in the cochlear. These damage seemed to have existed solely or combined showing the complexity of SD etiology.

PLASMA INTERLEUKIN 8 LEVELS ARE INCREASED BY HEMODIALYSIS

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Interleukin (IL)-8 acts as a chemotactic and stimulating factor for neutrophils, T-lymphocytes and basophils. Thus, IL-8 plays an important role in inflammation. Most uremic patients treated with hemodialysis (HD) suffer from immediate inflammatory complications such as hypotension, fever and pruritus during HD sessions. The release of cytokines from inflammatory cells during HD may be involved in such complications. The possibility that IL-8 production was influenced by dialzers was studied as follows. Plasma IL-8 levels and the expression of IL-8 mRNA in peripheral blood mononuclear cells (PBMC) were determined by utilizing the blood samples at the start and the end of HD. Seven kinds of dialyzers were evaluated in uremic patients undergoing maintenance HD. Plasma IL-8 levels in the patients after HD sessions with RC, PMMA, EVAL or PA dialyzer were significantly higher than those before HD. In contrast, plasma IL-8 levels did not change after HD with PAN, PS, or CTA. An increase in the expression of IL-8 mRNA in PBMC after HD with each dialyzer was compatible with that in plasma IL-8 level. Incubation of IL-8 with each membrane demonstrated that IL-8 was absorbed only by PAN. The present study demonstrates that evaluation of plasma IL-8 levels before and after HD sessions is useful as a new index for biocompatibility of dialyzers.

EVIDENCE FOR NONCLONAL HEMATOPOIETIC PROGENITOR CELL POPULATIONS IN BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Clonality of marrow hematopoietic progenitor cells in myelodysplastic syndromes (MDS) was analyzed by X-chromosome inactivation pattern using polymerase chain reaction (PCR). Five female patients were included in this study; two with refractory anemia (RA) and three with RA with excess blasts (RAEB). They were heterozygous for *Bst*XI restriction fragment length polymorphisms of the X-chromosome-linked phosphoglycerate kinase (*PGK*) gene. In each patient, erythroid and nonerythroid colonies, grown in the presence of erythropoietin and granulocyte-macrophage colony-stimulating factor (GM-CSF), exhibited no remarkable difference in clonal constitution. Two patients showed only one methylation pattern, suggesting the monoclonal origin of hematopoietic progenitor cells. Colonies of two other patients exhibited predominant and minor methylation patterns in *PGK* gene, indicating that nonclonal progenitor cells remain as a minor population. The bone marrow of one patient appeared to contain a greater proportion of nonclonal progenitors. Stem cell factor (SCF), a potent colony-stimulating factor, enhanced both erythroid and nonerythroid colony formation. However, it did not notably alter the clonal constitutions. Comparison of clonality between circulating polymorphonuclear cells and either T lymphocytes or mononuclear cells suggested that acquired clonal evolution caused the skewed constitutions of marrow hematopoietic progenitor cells. We thus conclude that nonclonal hematopoietic progenitor cells can persist in a substantial number of MDS patients.