ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

Nagoya, December 1994

Abstracts

FATAL EPSTEIN-BARR VIRUS-ASSOCIATED LYMPHOPROLIFERATIVE DISORDER IN CHILDHOOD

YASUSHI YATABE

1st Department of Pathology

Objective.—We report five childhood cases of fatal Epstein-Barr virus (EBV)-associated lymphoproliferative disorder to determine their immunopathologic and immunogenotypic features.

Design.—Clinicopathologic features are described, using clinical, histologic, immunophenotypic, and genotypic examinations.

Setting.—Autopsy cases which were performed at Nagoya and Tsukuba university hospitals, Japan.

Results.—Infiltrating lymphocytes, which were positive for the EBV genome by in situ hybridization, were polymorphic, and showed polyclonal immunoglobulin staining in all the cases. Two cases, however, showed genetic clonality of EBV termini. In one case, the clonality was observed only in the spleen, and in the other case, clonal rearrangement of the JH gene was also found. The former case showed none of the morphologic features of neoplasia and presented a morphology similar to virus-associated hemophagocytic syndrome. In the latter case, infiltrating lymphoid cells were much less polymorphous, and nodular masses of the lymphoid cells in colon and lung specimens were observed.

Conclusion.—We suggest that some polyclonal EBV-associated lymphoproliferative disorders develop into polymorphic, but genotypically monoclonal, lymphoproliferative disorders.

EFFECT OF DAILY VOLUNTARY RUNNING ON IN VIVO INSULIN ACTION IN RAT SKELETAL MUSCLE AND ADIPOSE TISSUE AS DETERMINED BY THE MICRODIALYSIS TECHNIQUE

WEI-PING YANG

1st Department of Health Promotion Medical Science

The effect of physical training on in vivo insulin-stimulated glucose utilization in relation to glycolysis (lactate formation) in rat peripheral tissues was investigated in 8 sedentary controls (SC) and 7 voluntary running rats (VR). We used a sequential euglycemic clamp procedure (insulin infusion rate: 6.0, 30.0-mU/kg · min) in combination with a microdialysis technique in M. quadriceps femoris, vastus lateralis, and inguinal adipose tissue. In the clamp study, glucose infusion rate (GIR) averaged over 45—75 min during the 6.0-mU/kg · min insulin infusion was significantly ($p < 0.01$) higher in VR (15.36 ± 0.83mU/kg · min, mean ± SE) than in SC (10.41 ± 0.88mU/kg · min), and the lack of a significant difference in GIR between VR and SC was found during the 30.0-mU/kg · min insulin infusion. In these tissues, there was no significant difference in dialysate lactate levels between VR and SC in the basal state without insulin

or glucose infusion, or at an insulin infusion rate of 30.0 mU/kg · min. However, dialysate lactate concentrations in muscle averaged over 45–75 min during the 6.0-mU/kg · min insulin clamp procedure in VR (8.51 ± 0.71mg/dl) were significantly ($p < 0.05$) higher than in SC (6.18 ± 0.48mg/dl). These results indicated that insulin action in skeletal muscle and adipose tissue could be evaluated in vivo by using the microdialysis technique, and that an increase in GIR in VR was, in part, explained by an increase in lactate formation in skeletal muscle.

INTRAPORTAL ENDOVASCULAR ULTRASONOGRAPHY IN THE DIAGNOSIS OF PORTAL VEIN INVASION BY PANCREATOBILIARY CARCINOMA

TETSUYA KANEKO

2nd Department of Surgery

Objective

The purpose of this study is to determine the value of intraportal endovascular ultrasonography (IPEUS) in the diagnosis of portal vein invasion by pancreatobiliary carcinoma. The authors report the results with this new technique and compare it to conventional imaging technologies such as portography and computed tomography (CT).

Summary Background Data

Pancreatobiliary carcinoma often invades the portal vein. By observing the echogenic band of the portal venous wall by a high-frequency, high-resolution intravascular ultrasound (IVUS) catheter, the portal vein in vasion can be accurately diagnosed.

Methods

A prospective study of 30 consecutive patients with pancreatobiliary carcinoma (16 pancreatic carcinomas, 8 bile duct carcinomas and 6 gallbladder carcinomas) was performed. In 23 cases IPEUS was performed intraoperatively from the superior mesenteric venous route with an 8 French, 20 MHz IVUS catheter. In 7 cases IPEUS was performed preoperatively from the percutaneous transhepatic route with a 6 French, 20 MHz IVUS catheter. The finding of IPEUS was confirmed by pathologic examination of resected specimens and surgican exploration. The results of IPEUS were compared to those of portography and CT.

Results

IPEUS visualized the portal venous wall as an echogenic band with a thickness of 0.5 mm to 1.0 mm. The diagnostic criterion of portal vein invasion was destruction of this echogenic band. Portal vein invasion was found in 15 of 30 cases. Vascular invasion was confirmed by pathologic examination of resected specimens in 10 patients and operative findings in 5. The sensitivity, specificity and overall accuracy of IPEUS for diagnosis of portal vein invasion was 100%, 93.3% and 96.7%. The values were 80%, 67.7% and 73.3% for portography and 53.3%, 80% and 66.7%, respectively, for CT.

Conclusions

IPEUS provided precise information about the relationship between the pancreatobiliary tumor and the portal venous wall. It was capable of accurately detecting or excluding early invasion of the portal venous wall by pancreatobiliary carcinoma.

LASER HIGH PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF PROSTAGLANDINS IN NASAL LAVAGE FLUID IN ALLERGIC RHINITIS

MASATO SUGIMOTO

Departments of Otorhinolaryngology

This study was designed to analyze prostaglandins (PGs) in human nasal lavage fluid using the combination of microcolumn high performance liquid chromatography and a He/Cd laser induced fluorescence detection system. Forty seven patients with allergic rhinitis and twelve healthy volunteers were investigated. Four species of PG, *i.e.*, PGD_2, PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ were detected in the nasal lavage fluid. Concentration of PGD_2 (1.33 ± 0.17 nmol/ml) and PGE_2 (0.87 ± 0.11 nmol/ml) in nasal lavage fluid from patients with allergic rhinitis (the allergy group) were significantly increased compared with those of volunteers (the control group, 0.23 ± 0.16 nmol/ml, 0.29 ±0.19 nmol/ml, respectively). On the other hand, no significant differences were observed in concentrations of either $PGF_{2\alpha}$ or 6-keto-$PGF_{1\alpha}$ between the control group and the allergy group. Histamine concentration in nasal lavage fluid was significantly increased in the allergy group (53 ± 7.6 nmol/l) compared with the control group (3.4 ± 1.0 nmol/l). No significant correlation was observed between PGD_2 and histamine concentration ($r = 0.24$), or between PGE_2 and histamine concentration ($r = 0.08$) in nasal lavage fluid from patients with allergic rhinitis. Treatment with oxatomide, an anti-histamine and anti-allergic drug, significantly improved symptom scores, but did not alleviate them completely. Concentrations of each PG detected in nasal lavage fluid did not change significantly after oxatomide treatment. It is concluded that, not only histamine but also PGs, particularly PGD_2 and PGE_2, might be involved in the genesis of allergic rhinitis.

IMMUNOHISTOCHEMICAL CHARACTERISTICS OF CHICKEN SPLEEN ELLIPSOIDS USING NEWLY ESTABLISHED MONOCLONAL ANTIBODIES

KENJI KASAI

1st Department of Pathology

Ellipsoids, the extra-vasculature sites surrounding penicilliary capillaries of the chicken spleen, play critical roles in the immune response and also in the clearance of pathogens or other particles. The meshwork of ellipsoids is formed by fibroblastic reticular cells. To characterize ellipsoidal reticular cells, a series of monoclonal antibodies against the chicken spleen have been developed. Of these antibodies, CSA-1 antibody reacts with fibroblastic reticular cells in ellipsoids and with endothelial cells. The reticular nature of positive cells in ellipsoids is indicated by immunoelectron microscopy, and by double-staining with anti-heat-shock protein

47kDa (hsp47) antibody. The reaction of CSA-1 with reticular cells is limited in ellipsoids; CSA-1 does not react with reticular cells in other lymphoid organs. These findings indicate that ellipsoidal reticular cells share the antigen with endothelial cells. Ontogenic studies reveal that, on embryonic day 18, the development of ellipsoids is completed, penicilliary capillaries become fenestrated, and CSA-1 expression in ellipsoids begins. These findings suggest that CSA-1 is expressed on the cell surface of ellipsoidal reticular cells once they are exposed to blood flow.

IMMUNOHISTOCHEMICAL DETECTION OF ENDOTOXIN IN ENDOTOXEMIC RATS

MOTOSHI YASUI

2nd Department of Surgery

Although there are various methods, including the use of radiolabeled LPS and specific antibodies, its distribution among the various organs is not well analyzed. The time course of the distribution of lipopolysaccharide (LPS) in the liver, spleen, lung, and kidney after intravenous (i.v.) or intraperitoneal (i.p.) injection of LPS was studied by immunohistochemical staining using a newly developed monoclonal antibody against Factor C in rats. Factor C, which is the initial activator of the Limulus clotting system, has a specific affinity for LPS. Moreover, plasma endotoxin levels were measured by a modification of a chromogenic endotoxin-specific assay. At 30 minutes after injection in the i.v. group and at 12 and 24 hours in the i.p. group, endotoxin was present on Kupffer cells by staining and on some sinusoidal endothelial cells in the liver as well as on macrophages in the marginal zone of the spleen. The plasma endotoxin levels in the i.v. group decreased gradually after injection. However, levels in the i.p. group gradually increased, reaching a maximum level at 6 hours after injection, and then gradually decreasing. Accordingly, these results suggest that, regardless of the route of injection, endotoxin can be detected by an immunohistochemical staining method using a monoclonal antibody against Factor C.

TISSUE DISTRIBUTION OF PROTEIN DISULFIDE ISOMERASE, ERp72, and ERp61

KEN-ICHI IIDA

Second Department of Pathology

Protein disulfide isomerase (PDI), ERp72, and ERp61 have thioredoxin-like domains and the COOH-terminal ER-retention signals and they are known to concern with protein folding in the endoplasmic reticulum. In order to investigate the systemic distribution of PDI, ERp72, and ERp61 in the rat tissue, we generated a monoclonal antibody against ERp72 as well as affinity-

purified polyclonal antibodies to ERps. Three proteins were distributed in broad variety of cell types. ERp61 showed similar pattern with ERp72 in most tissues except for testis, pancreas, pituitary gland, and lymphocyte. The strong reactions of the three proteins were recognized in follicular epithelia of the thyroid gland and, mucous glands of the tracheal and bronchial submucosa. Moderate expression was seen in bronchial epithelia, chondrocytes of tracheal cartilage, hepatocytes, and neurons. In the small intestine, the apical cytoplasm of surface epithelial cells of the villi contained moderate amounts of the three proteins, whereas immature goblet cells expressed ERp72 and ERp61 except PDI, on the contrary, Paneth cells showed staining for only PDI. PDI was abundant in pancreatic exocrine cells while it was scanty in pancreatic islet cells, however, ERp61 was more rich in islet cells than in exocrine cells. ERp72 was detected moderately both in islet cells and in exocrine cells. The staining of the plasma cells found in the interstitium of stomach, intestine, and salivary gland was very strong in the case of ERp72. The staining for ERp61 was relatively strong and that of PDI was weak. Accordingly, PDI family proteins might cooperate or divide up the work to play various roles.

DISTINCTIVE EXPRESSION OF E-SELECTIN ON THE ENDOTHELIAL CELLS OF SMALL VEINS AND PROLIFERATING VESSELS IN HUMAN COLORECTAL ADENOCARCINOMA

CHUN-LIN, YE

2nd Department of Surgery

It is said that selectin-carbohydrate interactions related to hematogenous spread of cancer cells. In this study, the pattern of E-selectin expression and correlation coefficient between E-selectin and sialyl lewis antigens were studied in human colorectal adenocarcinoma by immunohistochemistry. A total of 57 tissue specimens from 23 cases were collected, including primary colorectal adenocarcinoma, normal colorectal mucosa, hepatic metastasis of colorectal adenocarcinoma and hepatic tissue. A distinctive pattern of E-selectin expression on the endothelium of small veins was noted as well as E-selectin expression on the endothelium during the process of venous proliferation. The probability of correlation coefficient was <0.05 between E-selectin and sialyl Le^a, but not between E-selectin and sialyl le^x. These results indicate that E-selectin not only plays a predominate role in the adhesion of human colorectal adenocarcinoma together with sialyl Le^a, but relates to the angiogenesis during the development of colorectal carcinoma. Moreover, the density of staining for E-selectin was markedly higher in liver metastatic lesions than in primary colorectal carcinoma foci ($P<0.05$), and the serum level of E-selectin was also significantly elevated in the patients with liver metastases compared to those without metastases ($P<0.05$). These findings suggest that E-selectin is particularly involved in liver metastasis, and the serum E-selectin level may be a useful indicator in the diagnosis of liver metastasis in colorectal adenocarcinoma.

DURATION OF LIVER ISCHEMIA AND HEPATIC REGENERATION AFTER HEPATECTOMY IN RATS

HIROTAKA MARUYAMA

2nd Department of Surgery

Since an occlusion of the vascular inflow to the liver is a useful technique in liver surgery, a relation between ischemia and regeneration in the liver is particularly important. The purpose of this study was to evaluate the effect of ischemic duration on liver regeneration after massive hepatectomy. Animals were subjected to segmental liver ischemia. After 30, 60 or 90 minutes, nonischemic liver lobes were resected (70% hepatectomy). Hepatectomy without prior liver ischemia was performed in the control group. On the 1st, 3rd, 5th, and 7th days following hepatectomy, a BrdU labeling index was calculated as a marker of liver regeneration. AST, ALT, and liver adeninenucleotides were also measured. Although 30 minutes of liver ischemia resulted in higher peak AST and ALT levels, liver regeneration and ATP levels were significantly higher than in control animals. Ninety minutes of liver ischemia resulted in significantly lower liver regeneration and ATP levels compared with the other treatment paradigms. Liver regeneration and ATP levels were almost identical to control animals, in rats with 60 minutes of ischemia preceding hepatectomy. We conclude that liver regenarative capacities can tolerate significant ischemia and that ralatively brief periods of ischemia can even accelerate liver regeneration.

THE DISTRIBUTION OF NEURONAL CYTOPLASMIC INCLUSIONS IN MULTIPLE SYSTEM ATROPHY

KEN-ICHI SUGIURA

Department of Neurology

Neuronal cytoplasmic inclusions (NCIs) were demonstrated by silver staining (Gallyas staining) and light microscopy, in the central nervous system of 18 patients with multiple system atrophy (MSA) — 6 olivopontocerebellar atrophy (OPCA), 6 striatonigral degeneration (SND) and 6 Shy-Drager syndrome (SDS). We observed NCIs in cerebral cortex, putamen, pons, medul la oblongata and spinal cord, especially in putamen and pons of all patients with MSA. No NCI was observed in the neuron in the cerebellum and midbrain. The findings above mentioned are common to three subtypes of MSA. We did not find any NCIs in the neurons in other neurodegenerative disorders and non-neurogenic disorders. Our findings indicate that NCIs represent a special neuron alteration characteristic of MSA. It would also support the theory that OPCA, SND and SDS represent various manifestations of a single condition, i.e. MSA.

TWO PHENOTYPICALLY DISTINCT B LYMPHOCYTES ($IgM^{high}IgD^{low}$ and $IgM^{low}IgD^{high}$) IN CHRONIC GASTRIC ULCER IN THE RAT

NAOKI OHMIYA

2nd Department of Internal Medicine

Immunohistological staining of tissue sections has demonstrated heterogeneity in the phenotypes of the B cell. The splenic marginal zone in the rat is particularly well developed and surrounds the lymphoid follicle. Immunohistochemical staining with anti-IgM and anti-IgD antibodies demonstrated that marginal zone B cells are mainly $IgM^{+}IgD^{-}$ cells, whereas the follicular B cells are $IgM^{+}IgD^{+}$ cells. We determined the kinetics and phenotypes of infiltrating B cells in gastric ulcer in the rat induced by gastric injection of acetic acid (Day 0). Few B cells were found in the lesion in the early stages of ulceration. On Day 40, two kinds of B cells with phenotypes of $IgM^{high}IgD^{low}$OX33 $(CD45)^{+}$OX19 $(CD5)^{-}$ and $IgM^{low}IgD^{high}$OX33 $(CD45)^{+}$OX19 $(CD5)^{-}$ were scattered in the granulation tissue of open ulcers, but not in the healed scar tissue. On Day 180, those two kinds of B cells formed primary follicles in the granulation tissue of open ulcers, but were absent from the healed scar tissue. The $IgM^{high}IgD^{low}$OX33 $(CD45)^{+}$OX19 $(CD5)^{-}$B cell was considered to be identical to the marginal zone B cell of rat spleen. This phenotype of B cell might be associated with inflammatory process in chronic gastric ulcer.

COTRANSLOCATION AND COLOCALIZATION OF hsp40 WITH hsp70 IN MAMMALIAN CELLS

MITSUO YAMANE

Department of Oral Surgery

In response to elevated temperature, living cells induce a set of proteins, called heat-shock proteins (hsp). Near twenty hsps are identified in mammalian cells so far. A novel 40-kDa heat-shock protein hsp40 in mammalian cells has been recently identified to be a homolog of bacterial DnaJ protein. It has been shown that hsp70, one of the most abundant hsp in cells and a homolog of bacterial DnaK protein has a molecular chaperon activity.

Bacterial heat-shock proteins DnaK, DnaJ and GrpE have been known to act together as a chaperon system in protein folding, prevention of protein aggregation and renaturation of denatured protein, assembly and disassembly of protein complexes. In this report we investigated intracellular translocation and localization of hsp40 and hsp70 in some mammalian cells. Translocation kinetics of hsp40 during heating at mild temperature was almost the same as that of hsp70 in HeLa cells. Hsp40 colocalize with hsp70 in heat-shocked cells. Direct interaction of hsp40 with hsp70 was observed by immunoprecipitation methods. Also treatments of cells with

cytoskeleton-acting drugs had no effect on the heat induced translocation of hsp40 and hsp70 in NRK cells. These results suggest that hsp40 and hsp70 form a complex in the cytoplasm at normal temperature, and translocate together and colocalize in the nuclei and nucleoli upon heat-shock, and they may function co-operatively to repair denatured proteins under stress conditions in mammalian cells.

RAPID ACCUMULATION OF DELETED MITOCHONDRIAL DEOXYRIBONUCLEIC ACID IN POSTMENOPAUSAL OVARIES

TAKESHI KITAGAWA

Department of Obstetrics and Gynecology

To study the aging process in human ovary, we analyzed the accumulation of mitochondrial DNA mutation in ovary in women of various ages. Thirty-nine tissues from ovaries and abdominal muscles, ranging from those of a fetus to those of a 77 year-old woman, were collected at artificial abortion or gynecological surgery. Total DNA was extracted from these tissues, and parts of mitochondrial DNA were amplified via polymerase chain reaction. Through these experiments, we found a 4,977-bp deletion of mitochondrial DNA between ATPase8 and ND5 genes in ovarian samples obtained from menopausal and postmenopausal women and in all muscle samples. Quantitative analysis of the deleted mitochondrial DNA also showed that the accumulation of deleted mitochondrial DNA in the ovary occurs rapidly around the menopausal period while the accumulation found in abdominal muscle occurs gradually with aging. These results indicated that mitochondrial DNA deletion occurs in muscular tissues even at very young ages but that accumulation of the deletion in ovarian tissue starts at the menopausal period and may have a relationship to dysfunction of the ovary in aging.

EFFECTS OF INTRACELLULAR pH ON CALCIUM CURRENTS AND INTRACELLULAR CALCIUM IONS IN THE SMOOTH MUSCLE OF RABBIT PORTAL VEIN

SHIGEO IINO

1st Department of Internal Medicine

In single smooth muscle cells dispersed from the rabbit portal vein, effects of intracellular pH (pH_i) on Ca^{2+} channel currents were studied with the whole-cell clamp method using nystatin in the pipette. Changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were also measured with the fluorescent indicator, fura-2, together with the mechanical response in intact tissues. pH_i was

modified with ammonium chloride (NH_4Cl) and propionate. Intracellular alkalinization caused by an application of NH_4Cl (20 mM) markedly potentiated and acidification caused by propionate (20 mM) inhibited inward Ca^{2+} channel currents, without much change in the kinetics. Tension development induced by 60 mM K^+ was inhibited by NH_4Cl (20 mM) and potentiated by propionate (20 mM), whereas the peak $[Ca^{2+}{}_i]$ level reached during K^+ contracture was reduced in the presence of NH_4Cl and increased in the presence of propionate. Inhibition of Ca^{2+} uptake into sarcoplasmic reticulum had little effect on changes of Ca^{2+} channel currents and $[Ca^{2+}]_i$ caused by pH_i alteration. It was concluded that the modification of Ca^{2+} channel currents caused by pH_i was not directly related to the effects of pH_i on the mechanical response to excess K^+. The direct effects of pH_i on $[Ca^{2+}]_i$ and on contractile machinery are considered to be mainly responsible for the mechanical effect of pH_i.

CIRCADIAN AND ESTROUS CYCLE-DEPENDENT VARIATIONS IN BLOOD PRESSURE AND HEART RATE IN FEMALE RATS

HIROTO TAKEZAWA

1st Department of Internal Medicine

To determine whether cardiovascular functions are controlled by the endogenous circadian system and whether they change with the estrous cycle in female rats, we measured mean arterial pressure (MAP), heart rate (HR), and spontaneous activity (ACT) of female rats using an implantable radiotelemetry device and a computerized data collecting system. Under a 12:12-h light-dark (LD) cycle, these parameters exhibited daily rhythms that were entrained to the photic cycle. The patterns of the daily rhythms varied with estrous cycles, and variations were particularly marked in the proestrous stage. During the dark period of this stage, ACT levels were significantly higher, but HR was significantly lower than in other stages. Although the peak MAP occurred within 2 h after the onset of the dark phase in three of the estrous stages, it occurred around midnight in the proestrous stage. Such estrous cycle-dependent variations were eliminated by ovariectomy. The implantation of 17β-estradiol produced a gradual increase in MAP and an abrupt decrease in HR. During constant darkness, all three parameters were free running, maintaining the same internal phase relationships with each other as during LD cycles. These results indicate that daily variations in these parameters were controlled by the endogenous circadian oscillating system, and that estrogen may be responsible for these estrous cycle-dependent variations.

NUCLEAR MAGNETIC RESONANCE STUDY ON METABOLITES UNDER ISCHEMIA-REPERFUSION WITH DIMETHYL AMILORIDE IN ISOLATED RABBIT HEARTS

AKIRA KOIKE

Department of Thoracic Surgery

This study was designed to investigate the ionic and metabolic mechanisms for the protective effect of dimethyl amiloride (DMA) against ischemia-reperfusion injury in normothermic (37°C) isolated, perfused young rabbit hearts. After the equilibration, the control perfusate was switched to normothermic St. Thomas' Hospital cardioplegic solution for 2 min to make the heart arrest, then the hearts were subjected to normothermic (37°C) global ischemia for 45 minutes, followed by 40 minutes reperfusion with control perfusate as was used for equilibrium. In hearts treated with 10 μM DMA, mean percent recoveries of LVDP at 40 min reperfusion was significantly higher than that in control hearts (111 ± 6.1% versus 78.7 ± 3.5% (mean ± SEM)). Mean Intracellular pH (pHi) of the equilibrated hearts was 7.3 ± 0.04 in control group (n=8) and 7.3 ± 0.05 in treated group (n=6). Decrease in pHi during ischemia in treated hearts was significantly enhanced during middle to late ischemia (pHi reached 6.2 ± 0.04 versus 5.2 ± 0.16 at the end of ischemia in treated and control hearts, respectively). Changes in creatine phosphate, inorganic phosphate and ATP did not show any statistically significant differences between control and treated groups. In conclusion, these findings suggest that: 1) 10 μM of DMA shows a protective action against ischemia-reperfusion injury; 2) the protective action of DMA is concerned with the intracellular hydrogen accumulation (excessive intracellular acidosis) in ischemic period; and 3) the time courses of the changes in high energy phosphates were not affected by treatment with DMA and they showed no correlation with the left ventricular recovery.

LEWIS X STRUCTURE INCREASES CELL SUBSTRATUM ADHESION IN L CELLS

AKIHARU SUDOU

1st Department of Biochemistry

cDNAs of α-1,3-fucosyltransferase as well as α-1,3/4-fucosyl-transferase were placed under the control of a β-actin promoter and cytomegalovirus enhancer and were introduced into L cells. The trans-fected cells expressing Le^x antigen showed increased cell substratum adhesion as compared to the antigen negative cells, when they were cultured for 2 to 4 h in Dulbecco-modified minimum essential medium containing 0.05% bovine serum albumin. The increased cell substratum adhesion was completely inhibited by cycloheximide and anti-integrin antiserum, and partly by an RGD peptide and EGTA. These findings indicate that Le^x structure promotes

cell adhesion to substratum-bound material secreted by cells, and that the increased adhesion is mediated by integrin. Western bloctting experiments have revealed an 85 kDa protein and a 50–60 kDa protein as carriers of Le^x antigen in transfected cells. The latter is likely to be basigin, which is a member of the immunoglobulin superfamily and is considerd to be an integrin-associated protein. A hypothesis is proposed that fucosylation of basigin enhances integrin-mediated cell substratum adhesion.

STRUCTURE AND REGULATION OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE I (CAM KINASE I)

HISAYUKI YOKOKURA

Department of Pharmacology

CaM kinase I was originally identified in rat brain based on its ability to phosphorylate site 1 of synapsin I. Recently, a cDNA for the rat brain enzyme has been cloned and the primary structure elucidated. Based on the predicted sequence, a regulatory domain containing the CaM-binding region is suggested to be located near the COOH-terminus of the protein. The mechanism of regulation of CaM kinase I was investigated using a series of CaM kinase I COOH-terminal truncated mutants. Each mutant was expressed as a glutathione S-transferase fusion protein and purified using a glutathione-Sepharose affinity column. Wild-type CaM kinase I (residues 1-332) showed total dependence on CaM for its activity. In contrast, a mutant kinase (residues 1-293) lost the ability to bind CaM, but acquired the constitutive activity. These results suggest that the region corresponding to residues 294-the COOH-terminus contain the CaM-binding domain and the autoinhibitory domain. This is supported by the studies that the peptide encompassing residues 294-321 inhibited the CaM-dependent activity of wild-type kinase. Moreover, the peptide inhibited the constitutive mutant competitively with respect to the peptide substrate, syntide-2. Therefore, these results indicate that CaM kinase I is regulated through the intasteric mechanism.

INHIBITION OF ACID SECRETION IN GASTRIC PARIETAL CELLS BY THE Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II INHIBITOR KN-93

NAOTO MAMIYA

1st Department of Internal Medicine

Gastric parietal cell secretion is stimulated through both histaminergic and muscarinic cholinergic pathways. A selective inhibitor of CaM Kinase II, KN-62, inhibited carbachol-

stimulated aminopyrine uptake but did not significantly affect the histamine stimulation. A further potent inhibitor of CaM Kinase II, KN-93, has recently been synthesized. Both KN-62 and KN-93 elicited a potent inhibitory effect on CaM kinase II in a competitive fashion against calmodulin with Ki value of 0.9 and 0.37 μM, respectively. All three kinds of acid secretagogues, gastrin, acetylcholine and histamine, bring about an elevation of the intracellular calcium concentration. Therefore it is possible that an increase in the cytosolic free calcium concentration ([Ca2+]i) may influence intracellular events during secretagogue stimulation. Stimulated parietal cells undergo a morphological transformation during stimulation. Involvement of the multifunctional CaM kinase II during these processes has been implicated in parietal cells when stimulated through cholinergic pathway. We have investigated the effects of the potent CaM Kinase II inhibitor KN-93, on the intracellular events in the acid secreting parietal cells. A novel Ca2+/calmodulin-dependent protein kinase II (CaM Kinase II) inhibitor, KN-93, potently inhibits gastric acid secretion from parietal cells. On the other hand, treatment of parietal cells with a selective inhibitor of CaM kinase II, KN-62 resulted in the inhibition of cholinergic-stimulated rabbit parietal cell secretion, whereas it failed to inhibit the histamine and forskolin response. In contrast, effects of carbachol, histamine and forskolin were significantly inhibited by KN-93 with an IC50 of 0.15, 0.3 and 1 mM, respectively; these effects occurred without any changes in intracellular cyclic AMP and Ca2+ levels. In the present study we investigated the mechanism by which KN-93 acts upon the acid-secreting machinery of gastric parietal cells. Neither redistribution of the proton pump activity nor the morphological transformation was affected by KN-93. The drug only weakly inhibited the H+, K+-ATPase activity but strongly dissipated the proton gradient formed in the gastric membrane vesicles and reduced the volume of luminal space. Thus KN-93 act at pH gradient formation, whereas KN-62 acts only at CaM Kinase II.

EXPRESSION AND TYROSINE PHOSPHORYLATION OF PHOSPHATIDYLINOSITOL-3 KINASE IN HUMAN GASTRIC CANCER CELLS AND ITS CORRELATION WITH CELL GROWTH

HENGYI XIAO

Department of Molecular Pathogenesis, Institute for Disease Mechanism and Control

Phophatidylinositol-3 kinase (PI-3 kinase) is one of the key substrates of tyrosine kinase, and appears to be important for various signaling including mitogenesis, intracellular transport and cytoskeletal changes. To study its role in the oncogenecity of human cancer cells, we examined the expression, tyrosine phosphorylation and activity of PI-3 kinase in human gastric cancer cell lines, NUGC-4 and MKN-45, which showed aberrant elevated in vivo tyrosine kinase activities. The expression, tyrosine phosphorylation and overall activity of PI-3 kinase in these cells were apparently elevated, compared with those in one human fibroblast cell line and another one human gastric cancer cell line. Morever, the activities of PI-3 kinase in both of these cells were suppressed by a tyrosine kinase inhibitor, genistein, but their responses to another two tyrosine kinase inhibitors, and acid treatmant were different. We also found that treatmant of these cells with wortmannin, a potent inhibitor of PI-3 kinase, suppressed the growth of these gastric

cancer cells. Thus, these results suggest that the signaling via tyrosine phosphorylation is required for the activation of PI 3-kinase in both NUGC-4 and MKN-45 cells but the tyrosine kinases involved may be different in these two cells, and that PI-3 kinase plays an important role in the growth of NUGC-4 and MKN-45 cells.

A SUBSTRAIN OF NZB MOUSE AS AN ANIMAL MODEL OF AUTOIMMUNE INNER EAR DISEASE

MICHIHIKO SONE

Department of Otorhinolaryngology

A substrain of an autoimmune-prone mouse, NZB/kl, was found to show spontaneous elevation of the auditory brainstem response (ABR) threshold with age. Morphological examination of the inner ear in NZB/kl mice with high ABR thresholds revealed pathological changes confined to the stria vascularis, including marked thickening of the capillary basement membrane which contained many foamy structures, and vacuolar degeneration of the intermediate cells. Circular or granular IgM deposits and some IgG deposits were found in the stria vascularis in the mice with high ABR thresholds, suggesting that deposits of immune complexes (mainly IgM antibodies) could cause strial damage that resulted in the ABR threshold elevation. Another substrain of NZB mice, NZB/san, showed lower levels of IgM immune complexes and anti-ss DNA antibodies, and developed neither inner ear morphological changes nor a high ABR threshold. NZB/kl mice may provide a useful animal model for studying the mechanism of autoimmune inner ear disease.

SUPPRESSIVE ROD-CONE INTERACTION IN COMPLETE AND INCOMPLETE TYPE OF CONGENITAL STATIONARY NIGHTBLINDNESS

NAOKI TOMIDA

Department of Ophthalmology

Dark-adapted rods have a suppressive effect on cone response (suppressive rod-cone interaction: SRCI). This phenomenon has been applied to analyse pathological mechanism of congenital stationary nightblindness (CSNB), but the result was discrepant among the investigators. Alexander et al reported that two individuals with CSNB exhibit a normal SRCI, but Arden and Hogg reported an absence of SRCI in four cases of CSNB. We evaluated SRCI in complete and incomplete types of CSNB. SRCI was tested by measuring luminance threshold for flicker detection (685nm, 1.7° in diameter, 20Hz) across the horizontal meridian of the visual field

without and with full-field background illumination. In twelve normal subjects, a reduction of the flicker threshold was observed during presentation of the background. Five cases with complete CSNB showed normal SRCI but in one case we failed to detect SRCI except one tested locus. Similarly five cases of incomplete CSNB showed normal SRCI but in one case we failed to detect SRCI except one tested locus. These results may indicate that SRCI is normal in both complete and incomplete CSNB, but in some cases, SRCI is absent because of local retinal dysfunction.

EFFECT OF HEAD TILTING ON CYCLODEVIATION IN NINE DIAGNOSTIC POSITIONS OF GAZE IN NORMAL SUBJECTS AND CASES OF SUPERIOR OBLIQUE PALSY

HIDEKI NOMURA

Department of Ophthalmology

We measured byclodeviation in all nine diagnostic positions of gaze with the New Cyclo Tests, in 12 normal subjects and 5 patients with congenital superior oblique palsy. Measurements were carried out in the upright posture, and head tilt 15 or 30 degrees to the right or left. In normal subjects excyclodeviation was significantly greater in upward, right-upward and left-upward gaze than in primary position. The amount of excyclodeviation shows no difference between head tilt postures. It is suggested that effects of head tilting on cyclodeviation could be compensated by the correspondence on cyclotorsion of both eyes in normal subjects. In patient, four of the 5 cases showed no particular excyclodeviation in all nine diagnostic positions of gaze and in all head postures because of possible sensory adaptation. In one case, larger excyclodeviation was measured in upward, right-upward and left-upward gaze than in primary position. Excyclodeviation with head tilted to the right was greater than with other head postures. It is suggested that in some cases of superior oblique palsy excyclodeviation and increase of the hypertropia in head tilt test to the affected side are due to inferior oblique muscle overaction.

SENSITIVITY OF OSTEOINDUCTIVE ACTIVITY OF DEMINERALIZED AND DEFATTED RAT FEMUR TO TEMPERATURE AND DURATION OF HEATING

TAKAYASU ITO

Department of Orthopedics Surgery

For autogenous and allogeneic bone grafts, heat treatment has been thought to effectively kill malignant cells and viruses, such as human immunodeficiency virus (HIV). However, it is

unclear whether the heat treatment could preserve the bone-inductive activity. Cortical bones from 6 week-old rat femurs were heated in a water bath at temperature of 50−100°C for periods of 15 minutes − 10 hours. After the treatment, they were defatted and decalcified. Each sample was then implanted into the hamstring muscle of 3 week-old rats. Eleven days after the implantation, the samples were removed and mRNAs for alkaline phosphatase and collagens in the implant were determined. Twenty one days after the implantation, actual bone formation was studied by a histological analysis and the measurement of calcium content. Heat treatment at 60°C for 10 hours and 70°C for 1 hour preserved the bone-inductive activity as indicated by the induction of the mRNAs for alkaline phosphatase, type I and type II collagens. A significant decrease in type II collagen mRNA and calcium content was observed at 70°C when the implants were heated for 10 hours, suggesting the importance of evaluating the duration of heat treatment.

THE BIPOTENTIAL GLIAL PROGENITOR CELL-LINE CAN DEVELOP INTO BOTH OLIGODENDROCYTES AND TYPE II ASTROCYTES IN BRAIN

SIGEKI NOMURA

Department of Neurosurgery

Oligodendrocyte-type 2 astrocyte (O2A) progenitor cell *in vivo* might differentiate into oligodendrocytes. To examine the influence of the brain microenvironment on the differentiation, a mouse O2A lineage cell line, designated OS3 cells, were implanted into the mouse brain at various ages and regions. Injected into 1-week postnatal brain, OS3 cells expressed sulfatide, galactocerebroside (GalC), even myelin basic protein (MBP), which were not observed to be expressed in vitro. In contrast, in the over 6-month postnatal brain, many OS3 cells expressed glial fibrillary acidic protein (GFAP) and did not express much sulfatide/GalC. In the 1-month postnatal brain, OS3 cells differentiated to immature type of oligodendrocytes ($GalC^+$/$sulfatide^+$/MBP^-) as observed in the culture with serum free medium. Furthermore, OS3 cells showed a region-specific property in which oligodendrocyte markers were positive when cells were injected into the internal cortex, thalamus, corpus callosm and subventricle zone. In the hippocampus, however, $GFAP^+$ cells were predominant, although low expression of both glial markers was positive in the ventricle. These findings suggest that the differentiation of glial cells is controlled by regional and stage specific factors in the brain.

THE ATTENUATION OF SUPPRESSION OF MOTILITY BY TRIAZOLAM IN THE CONDITIONED FEAR STRESS TASK IS EXACERBATED BY ETHANOL IN MICE

KIYOYUKI KITAICHI

Department of Medical Pharmacy

In ordinary life, the use of hypnotics together with alcoholic beverages is very common, although there are no reports about the interaction between benzodiazepine-induced amnesia and alcohol. We investigated whether ethanol modified triazolam-induced amnesia in a conditioned fear stress task. When mice were placed 24 hours later in the same environment in which they had previously been exposed to an electric foot shock (training), they exhibited a marked suppression of motility (conditioned fear stress). Triazolam (0.01—0.1 mg/kg, s.c.) and chlordiazepoxide (5—10 mg/kg, s.c.), administered before training, attenuated the suppression of motility in the conditioned fear stress task in a dose-dependent manner, without affecting the sensitivity to an electric foot shock. These results suggest that both benzodiazepines may inhibit the process of acquisition of memory. Ethanol (1 g/kg, p.o.), which by itself, did not affect either suppression of motility or sensitivity to an electric foot shock, exacerbated the attenuation of the suppression of motility induced by both triazolam (0.01 mg/kg) and chlordiazepoxide (5 mg/kg). The effects of ethanol were completely antagonized by benzodiazepine receptor antagonist Ro 15—1788 (10 mg/kg, i.p.). These results suggest that ethanol may exacerbate the effects of benzodiazepines, mediated by $GABA_A$/benzodiazepine receptor complex.

COMPARATIVE STUDY OF THE NEW CLASS-III ANTIARRHYTHMIC AGENTS, MS-551 AND E-4031, ON ACTION POTENTIAL AND POTASSIUM CURRENTS IN SINGLE RABBIT VENTRICULAR MYOCYTES

CHENG JIAN HUA

2nd Department of Regulation of Organ Function

Comparative effects of MS-551 and E-4031 on the action potential duration (APD) and three repolarizing potassium currents, were investigated in single rabbit ventricular myocytes. At higher stimulation frequencies, the APD prolongation by MS-551 (10 μM) was enhanced, but that by E-4031 (1 μM) was attenuated. The APD prolongation by MS-551 (10 μM) decreased progressively as the rest duration increased, whereas that by E-4031 (1 μM) remained at the same level. Both MS-551 (10 μM) and E-4031 (1 μM) induced significant decreases in the outward rectifier potassium current (I_K), but induced no effects on the transient outward current (I_{to}) and the inward rectifier potassium current (I_{K1}). The intensity of the block on I_K by MS-551

progressively increased as the durations of depolarizing pulses were prolonged. At −75mV, recovery from the block by MS-551 was rapid with a time constant of 577 ± 179 msec (n=6). E-4031, however, did not produce any substantial recovery from the block even after 30 sec of rest. These findings suggest that MS-551 and E-4031 produce differing frequency dependencies in class-III action. This is presumably due to the different characteristics in binding kinetics to I_K channel between two drugs.

EFFECT OF C-PEPTIDE ADMINISTRATION ON WHOLE BODY GLUCOSE UTILIZATION IN STZ-INDUCED DIABETIC RATS

WEI WU

1st Department of Health Promotion Medical Science

Recent studies suggest that C-peptide stimulates glucose transport in isolated skeletal muscle. The effect of C-peptide on whole body insuin action in 25 streptozotocin (STZ. 60 mg/kg)-induced diabetic and 22 normal rats was studied using the euglycemic clamp procedure and continuous infusion of somatostatin (1.0 μg/kg/min). Plasma insulin levels during the 6.0- and 30.0-mU/kg/min insulin infusions rose to 60—90 μU/ml and 500—700 μU/ml, respectively. Blood glucose concentrations were clamped at the basal levels (3.7 mmol/l) in the normal rats and at 7.8 mmol/l in the diabetic rats. Biosynthetic human C-peptide (0.5 nmol/kg/min) was infused in 12 diabetic and 11 normal rats, resulting in concentrations of 20—40 nmol/l. The metabolic clearance rate of glucose (MCR) for the diabetic rats receiving C-peptide was significantly higher than those in the diabetic rats given saline at low-dose insulin infusion (12.0 ± 1.0 vs 6.3 ± 0.7 ml/kg/min. $p < 0.01$). There was no significant difference in MCR (12.7 ± 0.8 vs 12.2 ± 1.9 ml/kg/min) at high-dose insulin infusion between the diabetic rats with C-peptide infusion and saline infusion. No significant difference in MCR at low -(18.1 vs 1.1 vs 18.5 ± 1.1 ml/kg/min) and high-dose insulin infusions (38.2 ± 2.3 vs 37.7 ± 3.4 ml/kg/min) was found between the normal rats given C-peptide and saline. These results demonstrate that C-peptide has the capacity to increase glucose utilization in STZ-induced diabetic rats.

INCREASED ALANINE UPTAKE AND LIPID SYNTHESIS FROM ALANINE IN ISOLATED HEPATOCYTES OF WISTAR KYOTO FATTY RATS: INHIBITORY EFFECTS OF BIGUANIDES

KOICHI MORI

3rd Department of Internal Medicine

A genetically obese-hyperglycemic model, the Wistar Kyoto (WKY) fatty rat is thought to be a good animal model for studying metabolic characteristics of human NIDDM. Altered amino acids metabolism has been recognized to be one of the characteristic metabolic abnormalities in diabetes mellitus as well as hypeglycemia and hyperlipidemia. We measured alanine uptake into isolated hepatocytes of WKY fatty and lean rats to examine the pathophysiological characteristics of NIDDM. Lipid synthesis from alanine in hepatocytes was increased in fatty rats at 5 and 12 wks of age compared with lean rats although alanine uptake into hepatocytes was increased in fatty rats only at 12 wks. 0.5 ~ 3 mM metformin and 0.1 ~ 3 mM buformin inhibited lipid synthesis from alanine in hepatocytes. These observations suggest that lipid synthesis from alanine in hepatocytes of WKY fatty rats is accelerated prior to the onset of diabetes mellitus and it might cause the insulin resistance and that the inhibitory effect on increased lipid synthesis is one of the pharmacodynamic actions of biguanides.

PHYSIOLOGICAL CONCENTRATION OF ESTRADIOL INHIBITS POLYMORPHONUCLEAR LEUKOCYTE CHEMOTAXIS VIA A RECEPTOR MEDIATED SYSTEM

IZUMI ITO

Department of Geriatrics

Estrogen exhibits a variety of actions, including immuno-modulatory effects, in vivo and in vitro. The mechanism by which estrogen exerts its anti-inflammatory effect is not yet understood. We investigated the possible mechanisms of estradiol acting via the polymorphonuclear leukocytes (PMNs), which are important in the immune response. The agent, 17β-estradiol ($10^{-15} \sim 10^{-6}$ M), but not 17α-estradiol, significantly reduced PMNs chemotaxis to FMLP in a dose-dependent manner (control vs estrogen $10^{-13 \sim -11}$M, $P < 0.05$; $10^{-10 \sim -6}$M, $P < 0.01$). Physiological concentrations of estradiol significantly reduced the chemotaxis of PMNs (10^{-9} mol, 20% decrease). Pre-incubation with clomiphene or tamoxifen which are estrogen receptor antagonists, eliminated the inhibitory effect of 17β-estradiol on the chemotaxis of PMNs, restoring it to the control level. These observations suggest that 17β-estradiol suppressed the chemotaxis of PMNs by a receptor-dependent mechanism. In addition, the level of estradiol in human plasma, which PMNs were drawn, showed a close, inverse correlation with the PMNs chemotaxis to FMLP ($r = -0.821$ $p < 0.001$). Estrogen may modify the activity of neutrophils during

the normal menstrual cycle, not only during pregnancy, and influence inflammation.

EFFECTS OF PHOSPHODIESTERASE INHIBITORS ON SPONTANEOUS ELECTRICAL ACTIVITY, SLOW WAVE, IN THE GUINEA-PIG GASTRIC MUSCLE

MASANORI TSUGENO

Department of Neurosurgery

The circular muscle of the antral region of the guinea-pig gastric muscle generates rhythmic slow depolarizations of the plasma membrane, called slow wave, which are accompanied by contractions. Membrane potential in isolated muscle strips were measured using microelectrodes. To investigate the regulation of slow wave, we examined effects of xanthine derivatives and other cyclic AMP-related agents. Caffeine (0.1−1 mM), theophylline (0.3−3 mM) and IBMX (1−5 μM) reduced the frequency of slow wave without any clear changes in the membrane potential and configuration of slow wave. The membrane potential-independent inhibitory effects of these drugs were confirmed by changing extracellular K^+ concentration. Isoprenaline (3−10 μM), forskolin (30−300 nM), dibutylyl-cyclic AMP (200−500 μM), 8-bromo-cyclic AMP (200−500 μM) all produced effects essentially similar to those of xanthine derivatives. However, the inhibitory effects of xanthine derivatives were relatively more potent than other cyclic AMP related agents. Xanthine derivatives are thought to increase not only cyclic AMP but also cyclic GMP. The difference in the potency between the two groups of drugs may be due to the involvement of cyclic GMP. Slow wave appears to be a integrated electrical activity of a group of muscle cells. Xanthine derivatives may block intercellular coupling through changes in cyclic nucleotides.

PHOSPHATIDYLINOSITOL INHIBITS DNA POLYMERASE ε

MAMI SHOJI-KAWAGUCHI

Department of Cancer Cell Biology

We studied the effects of various phospholipids on the DNA synthesizing reactions by calf thymus DNA polymerase α, δ, and ε. Among three enzymes, DNA polymerase ε was most sensitive to acidic phospholipids, *i.e.,* phosphatidylinositol (PI), phosphatidylinositol-4-monophosphate (PIP), phosphatidylserine (PS), phosphatidic acid (PA), and cardiolipin (CAR). Among these acidic phospholipids, PI (from bovine liver) is of special interest because it inhibited DNA polymerase ε in a dose-dependent manner, while it did not affect DNA polymerase α and δ. The inhibition was competitive with the DNA template-primer and noncompetitive with dTTP substrate. The Ki value was estimated to be 16 μM in final concentration. Electrophoretic

analysis of reaction product revealed that PI decreased the frequency of initiation as well as the processivity of DNA polymerase ε reaction. These results indicate that PI from bovine liver can be used as a specific inhibitor for DNA polymerase ε to analyze its role in DNA replication. Interestingly, the PI isolated from soybean, which has a different fatty acid composition, inhibited not only DNA polymerase ε but also DNA polymerase α in the same manner.

INHIBITION OF TYPE A AND B MONOALMINE OXIDASE BY β-CARBOLINES AND N-METHYLATED β-CARBOLINIUM IONS

SHINNICHI HASEGAWA

Department of Neurology

The inhibition of type A and B monoamine oxidase from human brain mitochondria by β-carbolines (BC) and their N-methylated β-carbolinium ions were examined using 9H-pyrido[3,4-b]-indole (norharman), 1-methyl-9H-pyrido[3,4-b]-indole (harmane), 7-methoxy-1-methyl-9H-pyrido[3,4-b]-indole (harmine), 4,9-dihydro-7-methoxy-1-methyl-3H-pyrido[3,4-b]-indole (harmaline) and their N-methylated derivatives. All of them inhibited type A oxidase, and only norharman inhibited type B oxidase. Harmine and 2-methyl-harmine inhibited type A oxidase noncompetitively to the substrate, kynuramine, whereas the other compounds competitively. 2-Me-norharman is more potent inhibitor than norharman, which had been proved to be enzymatically methylated on the 2-N position and, sequentially on 9-N position to yield 2,9-Me_2-norharman in mammalian brain, and then 2,9-Me_2-norharman is more potent inhibitor than 2-Me-norharman. The types of their inhibition were competitive way. BCs have been known to have a phychiatric activity, and these results suggested that N-methylation of BCs in human brain possibly involved in the biological function of central nervous system, regulating the levels of cathecholamines and serotonine in the brain.

PHOSPHORYLATION OF RABPHILIN-3A, A PUTATIVE TARGET PROTEIN FOR *RAB*3A, BY CYCLIC AMP-DEPENDENT PROTEIN KINASE

SHIN-ICHIRO NUMATA

Department of Pediatrics

*Rab*3A, a member of the *Rab* family small GTP-binding proteins, is implicated in neurotransmitter release. Available evidence suggests that cyclic AMP-dependent protein kinase (protein kinase A) regulates neurotransmitter release. Therefore, it is important to investigate the relationship between protein kinase A and *Rab*3A in neurotransmitter release. Then, we examined

whether *Rab*3A and its associated proteins, such as Rabphilin-3A, a putative target protein for *Rab*3A, MSS4, a stimulatory GDP/GTP exchange protein for *Rab*3A, and *Rab*GDI, a translocator for the *Rab* family members including *Rab*3A are phosphorylated by protein kinase A. Neither *Rab*3A, MSS4, nor *Rab*GDI was phosphorylated, but Rabphilin-3A was stoichiometrically phosphorylated at its N-terminal region. About 0.8 mol of phosphate was maximally incorporated into one mol of Rabphilin-3A. The Km and Vmax values for the phosphorylation of Rabphilin-3A by protein kinase A were 1.8 μM and 7.2 pmol/min/mg of protein, respectively. Rabphilin-3A associated with the synaptic vesicles was also phosphorylated by protein kinase A. These results suggest that Rabphilin-3A is one of the targets for protein kinase A which modulates neurotransmitter release.

A NOVEL PROTEIN ACTIVATOR OF Ca^{2+}/CALMODULIN DEPENDENT PROTEIN KINASE I PARTIALLY PURIFIED AND CHARACTERIZED FROM RAT BRAIN

SHIGEO INOUE

Department of Neurosurgery

A novel activator of Ca^{2+}/calmodulin (CaM)-dependent protein kinase I was partially purified from rat brain. The activator was autophosphorylated in a renatured gel, indicating that the activator was a protein kinase. And the activation of CaM kinase I by the activator was detected in a renature gel and *in vitro*. The apparent molecular weight of the activator was 64 KDa. The recombinant glutathione S-transferase/CaM kinase I fusion (CaM kinase I) which was expressed in bacteria had a low CaM-dependent phosphorylating activity using the synthetic peptide syntide-2, as a substrate. The phosphorylation of CaM kinase I by its activator resulted in its drastic potentiation of the CaM-dependent enzymatic activity. We determined the changes of kinetic parameters of recombinant CaM kinase I by the activator. The activation decreased the Km values of the CaM kinase I for syntide-2 and ATP without changing the Vmax values. These results suggested that the activation mechanism through the phosphorylation of CaM kinase I by the activator might play a key role in the physiological function of CaM kinase I.

CALCYCLIN AND CALVASCULIN EXIST IN HUMAN PLATELETS

YASUHIRO TOMIDA

Department of Thoracic Surgery

Many of the platelet functions are tentatively explained to be mainly regulated by Ca^{2+}-calmodulin dependent phosphorylation of myosin light chain catalyzed by myosin light chain kinase. But the existence of intricate network in signal transduction, that is made up of interacting

systems regulated not only by calmodulin but also by various kinases, has been suggested with the progress in studying Ca^{2+}-signal transduction system in platelets. Calmodulin antagonists are proven to be good materials as affinity ligands to purify calmodulin and other Ca^{2+}-binding proteins. In this study, using Ca^{2+}-dependent affinity chromatography on a synthetic compound (W-7)-coupled Sepharose column, three distinct Ca^{2+}-binding proteins have been identified in human platelets. The molecular mass of these three distinct proteins were estimated 10, 10.5, 17 kDa by polyacrylamide-gel electrophoresis in the presence of SDS. The partial amino acid sequence revealed these proteins have EF-hand structures and high homology to the predicted proteins, each to calcyclin, calvasculin, and calmodulin. Calcyclin and calvasculin, a member of "S-100" protein family, have been considered probably playing roles in the control of the cell proliferation, but the existence of these two proteins in platelets suggests that they have other intracellular functions relating with Ca^{2+}-signal transduction system.

EFFECT OF GASTRIN-RELEASING PEPTIDE (GRP) ON GUINEA PIG GALLBLADDER CONTRACTION IN VITRO

FENG LIU

Department of Health Promotion Sciences

Few studies have been reported on the effects of gastrin-releasing peptide (GRP)/bombesin on the guinea pig gallbladder and the results are contradictory. We investigated the effect of GRP on guinea pig gallbladder smooth muscle using an improved horizontal organ bath. The guinea pigs were killed and the gallbladder was removed. Four longitudinal muscle strips (2 × 12 mm) were suspended in Krebs-Ringer solution at 37°C and aerated with 95% O2 and 5% CO2. The mechanical activities of the strips were recorded isotonically by displacement-voltage transducers via L-arms, to which a piezo electric element with a frequency of 100 Hz and movement of 50 mm was applied. GRP contracted gallbladder muscle strips dose dependently, but the calculated maximal response was 22.4% and 20.1% of acetylcholine and cholecystokinin (CCK) octapeptide, respectively. GRP-induced contraction was unaffected by a muscarinic blocker, atropine, or the CCK receptor antagonist, loxiglumide. From these results, it is concluded that GRP is a weak stimulant of guinea pig gallbladder contraction which it seems to stimulate directly.

STRUCTURE OF THE GENE ENCODING THE α SUBUNIT OF THE HUMAN INTERLEUKIN-3 RECEPTOR

HIROSHI KOSUGI

1st Department of Internal Medicine

Interleukin-3 (IL-3) is produced by T cells and macrophages and introduces hematopoietic stem cells to proliferate and differentiate. The receptor for IL-3 is composed of at least two subunits, α and β. In addition to the conserved cystein residues and a "WSxWS" motif, the extracellular segments of both subunits have domains that are structurally related to a fibronectin type III domain. This structure is conserved in all members of the cytokine receptor superfamily. We isolated and characterized genomic DNA clones containing the coding sequences of the α subunit of the human IL-3 receptor (hIL-3Rα). The gene spans approximately 50 Kb. 13 exons are included as is the same as the gene of the α subunit of the human GM-CSF receptor. We also determined the sequence of the upstream region about 500 b.p. of the hIL-3Rα gene and found the striking homology between part of the 5'-flanking region of the hIL-3Rα gene and the region about 1 Kb upstream of the human erythropoietin receptor. The putative promoter region has homologous sequence to binding site of Sp1 and GATA, and also contains notable purine-rich sequences. We compared the exon-intron organization of the hIL-3Rα gene with other members of the cytokine receptor superfamily and found the genomic organizations to be remarkably well conserved (2-1-0-1 rule). On the basis of these observations, we propose a model that this gene family evolved from a common ancestral gene.

DIVERSITY OF TANDEMLY REPETITIVE SEQUENCES DUE TO SHORT PERIODIC REPETITIONS IN THE CHROMOSOMES OF *CANDIDA ALBICANS*

HIROJI CHIBANA

Laboratory of Medical Mycology

In a previous study a repeated sequence, RPS1, was cloned from the genomic DNA of *Candida albicans*. It was 2.1 kb in length and was tandemly repeated in a limited region of almost all the chromosomes. In this study, we examined and characterized the diversity of the repeating structure of the RPS units. RPS units were of 2.1, 2.3, 2.5, and 2.9 kbp in length after digestion of the genomic DNA with *Sma*I, and 2.1 and 2.3 kbp after digestion with *Pst*I, with the differences being multiples of approximately 0.2 kbp. Moreover, one or two types of RPS unit were present specifically on each chromosome. We cloned 14 RPS units from the mixed DNA of chromosomes 1 and 2, and 59 RPS units from chromosome 6. These RPS units were classified into four types by their *Sfi*I-digestion profiles and chromosomal origins. Sequence comparisons revealed a tandem arrangement of internal, small repeating units of 172 bp. This unit of

repetition was designated *alt* (*C. albicans* tandem repeating unit). The size of RPS units was variable, with sizes representing a series of increments of approximately 0.2 kbp that corresponded to the *alt* sequence. By contrast, the sequences other than the tandem repeats of *alt*s were highly conserved, with homology of more than 98% among all cloned RPS units. These results suggested that RPS plays an important role in the organization and function of the chromosomes of *C. albicans* even though the actual function of RPS has not yet been clarified. Structural features of RPS that contains the repeated *alt* sequence are discussed in relation to human α-satellite DNA with its tandem repeats of about 170 bp that are similar in size to *alt*, the repetition of which is responsible for the variations in the size of the higher-order repeats.

ANALYSIS OF THE CHROMOSOMAL LOCALIZATION OF THE REPETITIVE SEQUENCES (RPSs) IN *CANDIDA ALBICANS*

ARIYA CHINDAMPORN

Laboratory of Medical Mycology

On the karyotyping studies in *Candida albicans*, a repetitive sequence (RPS1) was found and cloned from genomic DNA (Iwaguchi et al., 1992). The sequences similar to RPSI (so called RPS family) were isolated from some different chromosomes. It was demonstrated that their diversity was due to the presence of short periodic repetitions (Chibana et al., 1994). In this study, the location and the organization of repetitive sequences, members of the RPS family, that are sequences specific to *Candida albicans* were determined on each chromosome of *C. albicans* strain FC18. Using pulsed-field gel electrophoresis (PFGE), eight chromosomes were separated into seven fractions. Each chromosome was cleaved by *Bam* HI and *Xho* I to excise the RPSs, which were then detected by hybridization with an RPS probe. All chromosomes except chromosome 4 carried RPSs, and these RPSs were located within a limited region on each chromosome. From the digestion of each chromosome with *Sfi* I and probing with the RPSs, it was found that these recognition sites within the RPS region were conserved among all RPS containing chromosomes. For further characterization of the RPSs, the locations and the boundary regions of the RPSs on chromosome 6 of strain FC18 were examined as a model chromosome. Using the restriction enzymes *Sfi* I, *Sma* I, *Xho* I, *Bam* HI, *Mlu* I, and *Nru* I, a semi-macro physical map of the RPSs and their boundary regions on this chromosome was constructed. Which RPS part was adjacent to each boundary was determined by using subfragments of RPS as probes. The physical configuration around the RPSs and their boundary regions were presented. The results obtained here should be very useful for future analysis of the function of these regions.

MECHANISM OF ACTIVATION OF THE *ret* PROTO-ONCOGENE BY MULTIPLE ENDOCRINE NEOPLASIA 2A MUTATIONS

NAOYA ASAI

2nd Department of Pathology

The *ret* proto-oncogene (c-*ret*) encodes a transmembrane tyrosine kinase that contains a cadherine-like structure in the extracellular domain. Germ line mutations of c-*ret* are associated with multiple endocrine neoplasia (MEN) 2A and 2B. MEN2A mutations involve cystein residues in the extracellular domain of c-*ret*. We introduced MEN2A mutations in c-*ret* and analyzed their functions. Mutant c-*ret* genes showed high transforming activity on NIH3T3 cells. The 170kDa Ret protein on the cell surface of transformed cells was highly phosphorylated on tyrosine and formed disulfide-linked homodimers. This result indicated that MEN2A mutations induced ligand-independent dimerization of the Ret protein on cell surface, leading to activation of its intrinsic tyrosine kinase. In addition to MEN2A mutations, we further introduced a mutation (D300K) in a putative Ca^{2+}-binding site of the cadherine-like domain. Mutant c-*ret* gene with both MEN2A and D300K showed drastically increased transforming activity. In transfectants with D300k mutant, very little of the 170kDa Ret protein was expressed, while expression of the 150kDa Ret protein retained in endoplasmic reticulum was not affected. This result also demonstrated that transport of the Ret protein to the plasma membrane is required for its transforming activity.

TRANSFECTION-INDUCED TUMOR NECROSIS FACTOR-α INCREASES THE SUSCEPTIBILITY OF THE HUMAN GLIOMA CELLS TO LYSIS BY LYMPHOKINE-ACTIVATED KILLER CELLS: CONTINUOUS EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 ON THE GLIOMA CELLS

TORU TAKAOKA

Department of Neurosurgery

To develope more effective adoptive immunotherapy, we transfected the human tumor necrosis factor-α (TNF-α) gene into human glioma cells, which were used as target cells. TNF-α is known to increase the expression of intercellular adhesion molecule-1 (ICAM-1) on the surface of glioma cells and the susceptibility of glioma cells to lymphokine-activated killer (LAK) cell cytolysis. We compared the expression of ICAM-1 induced by TNF-α generated by the TNF-α gene-transfected cells with that induced by exogenously added TNF-α. When the TNF-α gene was transfected into target glioma cells, the expression of ICAM-1 was detected on the cell surface from 3 days after the transfection and continued until at least 9 days. In contrast, it was

expressed only transiently in the case of exogenously added TNF-α. Also, the cytolytic activity of LAK cells induced by transfection-induced TNF-α was significantly stronger than that induced by exogenously added TNF-α. The increased susceptibility was quenched by anti-ICAM-1 monoclonal antibody. These data indicated that continuous expression of ICAM-1 induced by TNF-α gene transfection of glioma cells resulted in higher cytolytic activity of LAK cells.

ANALYSIS OF THE JOINING SEQUENCES OF THE t(15;17) TRANSLOCATION IN HUMAN ACUTE PROMYELOCYTIC LEUKEMIA: SEQUENCE NON-SPECIFIC RECOMBINATION BETWEEN THE *PML* AND *RARA* GENES WITHIN IDENTICAL SHORT STRETCHES

HITOSHI YOSHIDA

Department of Internal Medicine, Branch Hospital

Molecular analysis of the t(15;17) translocation in 70 patients with acute promyelocytic leukemia (APL) confirmed that the breakpoints of chromosome 15 were located in two regions of the promyelocytic leukemia (*PML*) gene: mainly introns 3 and 6, whereas the breakpoints of chromosome 17 were consistently in intron 2 of the retinoic acid receptor alpha (*RARA*) gene. To study the reason for the clustering of the breakpoints and the underlying mechanism of the chromosomal translocation, we characterized the joining sequences of der(15) and der(17) by polymerase chain reaction in samples from 8 patients with APL. There was no cluster of the breakpoints within the introns, and no consensus sequence-motif was found around them. One or nine extra nucleotides were inserted into two joining sites. There were identical stretches of one to seven nucleotides between the *PML* and *RARA* genes in the majority of the joining sequences. These data provide a potential model of the t(15;17) translocation: random DNA double strand cleavage, modification of DNA ends by enzymes including terminal deoxynucleotidyl transferase, and single strand base-pairing within identical short stretches. Furthermore, APL is developed only when the *PML* and *RARA* genes are rearranged within their restricted genomic regions and functional *PML-RARA* chimeric product is produced. This might lead to clustering of the breakpoints.

IMMUNOSUPPRESSIVE EFFECT OF ANTI-ICAM-1 AND ANTI-LFA-1 MONOCLONAL ANTIBODIES ON THE FREE AND VASCULARIZED SKIN ALLOGRAFTS DURING REJECTION

TETSUYA IWATA

Department of Plastic and Reconstructive Surgery

Immunosuppressive effect of treatment with anti-adhesion molecule antibody on the rejection of the free or vascularized skin allograft was investigated in rats. Lewis (LEW, RT1l) rats were used as donors, and Fisher (F344, RT1lvl) rats as recipients. When F344 rats were treated intraperitoneally with anti-intercellular adhesion molecule-1 (ICAM-1) mAb (1A29) (3mg/kg) and anti-leukocyte function-associated antigen-1 (LFA-1) mAb (WT.1) (3mg/kg) one day prior grafting and daily for nine days after grafting, the free skin graft from Lew were only slightly prolonged compared with that in control rats which were injected i.p. with daily dose of 6mg/kg of anti-TNP mAbs (H1-6-2) one day prior grafting and daily for nine days after grafting. [Mean survival time (MST) of the free skin graft was 11.2 ± 0.6 days in control group and 13.4 ± 0.3 days in 1A29+WT-1 treated group ($p < 0.01$), respectively.] On the other hand, the vascularized grafts were significantly prolonged in anti-ICAM-1 mAbs and anti-LFA-1 mAbs-treated F344 rats as compared with control rats. [Mean vascularized graft survival time was 14.2 ± 0.7 days in control group and 21.5 ± 1.9 days in 1A29+WT-1 treated group ($p < 0.002$).] Our results suggest that interaction with ICAM-1 and LFA-1 play more important role in rejection of vascularized skin allo graft than that of free skin allo graft.

DOWN-REGULATION OF T CELL PROLIFERATION IN RESPONSE TO SOLUBLE aCD3 THROUGH DEVELOPMENT OF aCD3-REDIRECTED CYTOLYTIC ACTIVITY AGAINST CO-STIMULATORY CELLS

TINT LWIN

Department of Immunology

$CD4^+$ T cells-depleted spleen cells ($CD8^+$ T cells) activated by soluble aCD3 mAb (aCD3) suppressed the proliferations of $CD8^+$ T-depleted spleen cells ($CD4^+$ T cells) and normal T cells in response to aCD3. Antigen-nonspecific cytolytic activity was induced in aCD3 activated $CD8^+$ T cells. The whole Ab but not (Fab')2 of aCD3 induced cytolytic activity. Down-regulation of CD3 expression and carrying-over of aCD3 were observed on the cell surface of Thy-1^+ cells stimulated with aCD3. aCD3-induced cytolytic activity was shown against tumour cell lines of syngenic macrophages and B cells but not allogenic T cells. aCD3-activated T cells suppressed directly plaque forming cells in SRBC-primed spleen cells during 4hr mixed cultures. Correspondingly, Ig^+ cells in $CD8^+$ T cell fractions stimulated with aCD3 decreased markedly after the peaked proliferation. These results indicated that aCD3 induced the aCD3-redirected

cytolytic activity in resting $CD8^+$ T cells which could inactivate non-T co-stimulatory cells. Proliferation of $CD8^+$ T cells in response to restimulation with aCD3 was partially restored by addition of co-stimulatory cells. The restoration was influenced by various conditions such as strength of cytolytic activity, times of restimulation and concentration of co-stimulatory cells. $CD8^+$ T cells did not produce the detectable IL-2 in response to restimulation with aCD3. These results provided evidence for a new pathway of down-regulation of T cell proliferation by aCD3-activated cytolytic $CD8^+$ T cells that suppressed co-stimulatory cell function by aCD3-redirected cytolysis and consequently inhibited IL-2 production required for proliferation.

SELECTIVE INHIBITION OF NEWCASTLE DISEASE VIRUS HN GENE EXPRESSION BY INTERFERON-β

ZHONG YU

Department of Molecular Pathogenesis

It was found that Interferon-β(IFNβ) selectively inhibited the accumulation of the Hemagglutinin-neuraminidase (HN) gene transcripts of Newcastle disease virus (NDV), a paramyxovirus, at both the primary and secondary transcription levels in HeLa cells under the conditions where it did not remarkablly affect the transcription of the other genes including the downstream L gene and the viral genome replication. This modulation by IFNβ of relative abundancies of the viral gene transcripts were faithfully reflected by the levels of respective viral proteins in cells, and resulted in progeny production reduced to about one third of the control. No such IFNβ-induced down regulation of expression of a particular gene was found for Sendai virus (another paramyxovirus), Vesicular Stomatitis virus (a rhabdovirus), and Influenza A virus and so far such selective inhibition has not been reported previously for any negative strand RNA viruses. Based upon these observations and nucleotide sequence features characteristic of NDV genome, we discussed special features of NDV gene expression including a regulatory mechanism unique to the HN gene expression and a new model for transcription of NDV, namely, the need of RNA polymerase entry to a secondary site, presumably, the intergenic region between the HN and L genes for L gene expression. These findings also appeared to provide an opportunity and evidence as well to reveal a new mode of antiviral action of IFN.

QUANTITATIVE ANALYSIS OF HERPES SIMPLEX VIRUS DNA IN THE CEREBROSPINAL FLUID OF CHILDREN WITH HERPES SIMPLEX ENCEPHALITIS

YOSHIHIRO ANDO

Department of Pediatrics

Herpes simplex virus (HSV) DNA in the cerebrospinal fluid (CSF) of children with herpes simplex encephalitis (HSE) was quantified and typed using the polymerase chain reaction (PCR) assay. During the acute phase, HSV-DNA was detected in the CSF of 13 patients with HSE, including 5 neonates. A restriction profile of the PCR products cleaved with the restriction enzymes XhoI and BglII showed that 2 neonatal samples were HSV-2, and the remainder were HSV-1. The amount of HSV-DNA in the initial CSF ranged from 10^2—10^5 copies/ml. A significantly greater number of HSV-DNA copies was detected in neonates than in older children (mean 3.9 vs 2.5, log10 copies/ml $p < .05$). Except for one patient, the amount of HSV-DNA decreased gradually with acyclovir therapy. These results show that a quantitative PCR assay is applicable not only to the diagnosis of HSE but also for monitoring the response to antiviral agents.

IMMUNOHISTOCHEMICAL STUDIES ON PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA/CYCLIN) IN HEPATOCYTES OF BILIARY ATRESIA -A USEFUL PARAMETER TO PREDICT CLINICAL OUTCOME

MOAZZEM HOSSAIN

Department of Pediatric Surgery

The hepatic lobulus was studied histologically and immunohistochemically using the monoclonal antibody for proliferating cell nuclear antigen (PCNA/Cyclin, a cell cycle related nuclear protein) in 27 biliary atresia(BA) patients and 6 normal infants. The study disclosed that the labeling index (LI) for PCNA positive hepatocytes was $37.21 \pm 17.75\%$ in BA patients and $3.14 \pm 1.5\%$ in normal infants ($P < 0.0001$). LI for PCNA positive cells was higher in the periportal area than the pericentral area ($p < 0.01$). LI was not related to the age of patients at the time of hepatic portoenterostomy compared in two groups divided at the age of 60 days. LI was $20.80 \pm 7.03\%$ in patients who cleared jaundice postoperatively and $48.49 \pm 13.43\%$ in patients having persistent jaundice ($p < 0.001$). Conventional histological studies of the same specimens showed common findings of BA of the liver such as hepatocellular degeneration, necrosis, inflammatory cell infiltration and giant cell transformation. Most of BA patients exhibited $8.94 \pm 13.55\%$ of giant cell transformation out of 1000 hepatocytes. Patients who exhibited high

population of giant cell transformation had an unfavorable outcome. Only 0.42% of giant cells were immunoreactive for PCNA. In conclusion, the PCNA expression of hepatocytes was closely related to the prognosis of BA patients, which could be thereby used as a prognostic marker (indicator).

A NEW MODEL FOR PANCREATICOBILIARY MALJUNCTION WITHOUT BILE DUCT DILATATION: DEMONSTRATION OF CELL PROLIFERATION IN THE GALLBLADDER EPITHELIUM

KENITIRO KANEKO

Department of Pediatric Surgery

Patients with pancreaticobiliary maljunction without bile duct dilatation (PBMWBDD) develop gallbladder carcinoma frequently. No models of PBMWBDD exist, and no previous studies have clearly demonstrated changes relating to carcinogenesis in the gallbladder. We, therefore, examined the cell kinetics of the gallbladder epithelium in a new experimental model of PBMWBDD. A cat model was produced by performing choledocho-pancreatic side-to-side ductal anastomosis in nine animals. Five cats, in which the choledocho-pancreatic ducts were exposed only, served as controls. After 6 months, the gallbladders of these cats were removed and stained with anti-proliferating cell nuclear antigen (PCNA) antibody (PC10, Dako). The labeling index (LI) was determined from the percentage of positive nuclei in three microscopic fields. The diameter of the common bile duct was not different between the models and the controls. In the models, the number of PCNA positive cells was significantly increased. The mean ($\pm$ standard deviation) PCNA LI was 28.1 $\pm$ 12.2% in the models and 4.3 $\pm$ 1.6% in the controls ($p < 0.01$). These studies clearly indicate that this model is suitable for studying PBMWBDD and that PBMWBDD has a prominent proliferating effect on the gallbladder epithelium, which may play an important role in gallbladder carcinogenesis.