ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

Nagoya, July 1998

Abstracts

EXPRESSION CLONING AND INTRACELLULAR LOCALIZATION OF A HUMAN ZF5 HOMOLOGUE

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We have concentrated attention on isolating novel autoantigens using sera from autoimmune disease patients. We isolated a cDNA encoding a human homologue of ZF5 (hZF5) for the first time, which has five Kruppel-like C2H2 type zinc fingers at carboxyl terminus and the BTB/POZ (poxvirus and zinc finger) at the amino terminus, using autoimmune sera from a patient with overlap syndrome (dermatomyositis and scleroderma). The BTB/POZ domain is highly conserved throughout metazoan evolution. Sequencing of the entire cDNA revealed an open reading frame (ORF) of 1349 bp with a deduced protein sequence of 449 amino acid residues and a calculated molecular weight of 51.3 kD. The deduced amino acid sequence of hZF5 is highly homologous to mouse ZF5 (99% identity) and chicken ZF5 (95% identity).

Immunofluorescence studies revealed that HA-tagged hZF5 transiently expressed in COS-7 cells showed the nuclear dot pattern in the BTB/POZ domain-dependent manner. hZF5 might be a novel autoantigen targeted by autoimmune sera.

CHARACTERIZATION OF RET-SHC-GRB2 COMPLEX INDUCED BY GDNF, MEN2A, AND MEN2B MUTATIONS

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We analyzed the intracellular signaling pathway through Ret activated by GDNF, MEN2A and 2B mutations. The results showed that all of them induced a signal transducing complex consisting of Ret, Shc, and Grb2 proteins. GDNF clearly activated a Ras-MAPK pathway in human neuroblastoma cells. Ret is expressed mainly as two isoforms that differ in the carboxyteminal sequence: a long isoform and a short isoform. The long isoform contains the consensus sequence for binding of the Shc PTB domain but not its SH2 domain, whereas the short isoform has the consensus sequence for binding of both domains. In vitro binding assay revealed that the long isoform of the MEN2A-Ret protein and both isoforms of the MEN2B-Ret protein bound preferentially to the Shc PTB domain. On the other hand, the short isoform of MEN2A-Ret bound to the PTB and SH2 domains. In neuroblastoma cells expressing both isoforms of Ret, its activation by GDNF also resulted in the binding of both domains. GDNF and MEN2A mutations activate Ret by inducing its dimerization, whereas the MEN2B mutation increases Ret catalytic activity without dimerization. Our results thus suggest that Ret dimerization might be required for binding of the Shc SH2 domain to the short isoform.

BIOLOGICAL PROPERTIES OF RET WITH CYSTEINE MUTATIONS CORRELATE WITH MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A, FAMILIAL MEDULLARY THYROID CARCINOMA, AND HIRSCHSPRUNG'S DISEASE PHENOTYPE

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We investigated the transforming activity of the *ret* proto-oncogene with a mutation in cysteine 609, 611, 618, 620, or 634 detected in patients with multiple endocrine neoplasia type 2A (MEN2A), familial medullary thyroid carcinoma (FMTC), or Hirschsprung's disease (HSCR). Of these cysteine mutations, codon 634 mutations are known to be correlated with the development of MEN2A, whereas codon 609, 618, or 620 mutations were detected in two-thirds of FMTCs and in several cases of HSCR. Analysis of a total 18 mutant *ret* genes revealed that codon 634 mutations showed the highest transforming activity. The activity of *ret* with the codon 609, 611, 618, or 620 mutation was approximately 3- to 5- fold lower than that of *ret* with a codon 634 mutation. In addition, different amino acid substitutions for the same cysteine displayed comparable transforming activity. The expression of the cell surface form of Ret codon 609, 611, 618, or 620 mutation was very low compared with that of Ret with codon 634 mutation, indicating that the former four mutations might impair transport of Ret to the plasma membrane, as observed for several HSCR mutations affecting the Ret extracellular domain. These results thus suggest that mutations in cysteine 609, 611, 618, or 620 may have the potential to develop HSCR in addition to MEN2A and FMTC.

ACTIVATION OF $pp60^{src}$ IS CRITICAL FOR UNI-AXIAL CYCLIC STRETCH INDUCED ORIENTING RESPONSE OF FIBROBLASTS

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Fibroblasts (3Y1) aligned perpendicularly to the stretch axis in response to uni-axial cyclic mechanical stretch (120% in length, 1 Hz). Concomittentaly with this orienting response, we observed that protein tyrosine phosphorylation of cellular proteins (molecular masses of approximately 70 kDa and 120–130 kDa) increased and peaked at 30 min in response to cyclic stretch. Immunoprecipitation experiments revealed that paxillin, $pp125^{FAK}$, and $pp130^{CAS}$ were included in the 68 kDa, and 120–130 kDa bands, respectively. Treatment with herbimycin A, a tyrosine kinase inhibitor, inhibited the stretch-induced tyrosine phosphorylation and the orienting response. The kinase activity of $pp60^{src}$ increased and peaked at 20 min after the oneset of cyclic stretch. Treatment of the cells with an anti-sense S-oligodeoxynucleotide (S-ODN) against $pp60^{src}$ inhibited the stretch-induced tyrosine phosphorylation and the orienting response. To elu-

cidate the role of $pp60^{src}$, we performed the same sets of experiments using c-src-transformed 3Y1 (C3Y1) fibroblasts. Cyclic stretch-induced orienting response was significantly faster in C3Y1 than in 3Y1. Thus, these results strongly suggest that cyclic stretch induces the activation of $pp60^{src}$ and that $pp60^{src}$ is indispensable for the tyrosine phosphorylation of $pp130^{CAS}$, $pp125^{FAK}$ and paxillin followed by the orienting response in 3Y1 fibroblasts.

INTERACTIVE FUSION OF THREE-DIMENSIONAL IMAGES OF UPPER ABDOMINAL CT AND FDG PET WITH NO BODY SURFACE MARKERS

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The aim of this study is to propose and validate a new method to make a fused image of CT and FDG PET in the upper abdominal area with no body surface markers. PET and CT were obtained from patients with pancreatic cancer (N=5) and mass forming pancreatitis (N=2). First, we determined the midsagittal plane from PET and CT data. From the difference in the location of the midsagittal planes, rotations of Y (from back to front) and Z axes (from foot to head) and X translation (from right to left) were calculated. An upper pole of the kidney was determined by PET and CT data. It showed Y and Z translations. The images of the three-dimensional data set were fused on a workstation. Reproducibility was assessed by examining randomly misaligned PET and CT data by examining sets. Pancreatic cancer and its lymph node metastasis were easily identified on fused images. In reproducibility assessment, the average error of the rotation was 0.77 degrees. The average errors of the translations were 3.43, 4.70 and 9.23 mm on the X, Y and Z axes, respectively. In conclusion, this PET/CT image registration technique is feasible and practical. It allows precise anatomical assessment of normal and abnormal FDG accumulation.

BREAST ULTRASONOGRAPHY: DIAGNOSTIC EFFICACY OF THE COMPUTER-AIDED DIAGNOSIS SYSTEM USING FUZZY INFERENCE

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We evaluated the performance of computer-aided diagnosis (CAD) system using fuzzy inference on breast sonography comparing that of radiologists. Seven radiologists interpreted 24 malignant and 30 benign cases. Six criteria (shape, border, halo, internal echoes, posterior echoes, and edge shadows) were scored using a 5-point rating scale. The output was described as

a real number of 0.0 to 1.0. The sensitivity of the radiologists, the CAD in six-criteria version and in four-criteria version were 63.1%, 82.1% and 78.0%, respectively. The specificity of them was 71.0%, 45.7% and 51.9%, respectively. No significant differences in the areas under the binormal ROC curve (A_z's) were found.

ADJUSTMENT OF CREATININE CLEARANCE IMPROVES ACCURACY OF CALVERTS FORMULA FOR CARBOPLATIN DOSING

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Carboplatin clearance depends on the glomerular filtration rate (GFR), and Calvert's formula has been frequently used to achieve a target area under the time versus concentration curve (mg ml^{-1} min). Creatinine clearance is a substitute for GFR when creatinine values are determined by Jaffé method, which is being replaced by the enzymatic method. When the enzymatic method is used, the corresponding creatinine clearance theoretically exceeds GFR, and the use of creatinine clearance as GFR in Calvert's formula results in overdosing of carboplatin accordingly. In this study, we have established a model for adjusting the creatinine clearance to offset this bias based on a relationship between creatinine values measured by the Jaffé and enzymatic methods: adjusted creatinine clearance (ml//min) = creatinine clearance (ml/min) × [serum creatinine (mg/dl)]/[serum creatinine (mg/dl) + 0.2]. Subsequently, we validated this model using the data from 35 lung cancer patients. Estimated clearances of carboplatin with the original equation (creatinine clearance + 25) were systematically higher than observed clearances [mean prediction error (MPE) ± standard error (SE) = 26 ± 5%]. This positive bias was corrected by the adjustment (MPE ± SE = 5 ± 4%). When the enzymatic method is used, the adjusted creatinine clearance should be used in Calvert's formula.

CLONALITY ANALYSIS OF REFRACTORY ANEMIA WITH RING SIDEROBLASTS: SIMULTANEOUS STUDY OF CLONALITY AND CYTOCHEMISTRY OF BONE MARROW PROGENITORS

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X chromosome inactivation and polymorphism of the human androgen receptor (*HUMARA*) gene has been applied for analyzing the clonality of blood cells. In the present study, the clonal relationship was investigated between peripheral blood polymorphonuclear cells (PMNCs) and marrow progenitor cells and the origin of ringed sideroblasts in patients with refractory

anemia with ring sideroblasts (RARS) by polymerase chain reaction (PCR) of *HUMARA* gene. The X-inactivation patterns of circulating PMNCs and T lymphocytes as well as individual granulocyte colonies grown *in vitro* from bone marrow cells were analyzed. The development of ringed sideroblasts in erythroid colonies by iron staining and their X-inactivation pattern were also examined. All three RARS patients showed monoclonal PMNCs. In granulocyte colonies, however, two different X-inactivation patterns were observed in all patients, indicating that non-clonal progenitor cells remained in bone marrow. All erythroid colonies consisted of ringed sideroblasts exclusively showed one pattern dominant in those of PMNCs. Our findings suggest that non-clonal progenitor cells persist in some RARS cases, that erythroid progenitors show mosaicism, and that ringed sideroblasts may be derived from an abnormal clone involved in the pathogenesis of this disease.

EYE TRACKING DEVICE COMPARISONS OF THREE METHODS OF MAGNETIC RESONANCE IMAGE SERIES DISPLAYS

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This study evaluated the effectiveness of three kinds of display methods for Magnetic Resonance (MR) image interpretation using an eye-tracking device. Seven radiologists interpreted head MR studies using a single monitor (17-inch, 1024 × 1280) in the 4 images/screen display format. Three paging modes were compared: (A) rapid paging only; (B) multiple image series display at the same slice position with consecutive rapid paging, and (C) simultaneous display of multiple series with each image series being browsed independently. Using an eye-mark camera, the radiologist's point of fixation and the duration of fixation were recorded during actual image interpretation. In mode A, the duration of fixation was short, and the points of fixation were distributed randomly over the visual field. In mode B, the points of fixation were clustered chiefly on a specific image series. In mode C, the points of fixation were not clustered on a specified series, but the duration of viewing the T2 series was relatively long. The total tracing area in mode B and C was smaller than that in mode A. Multiple series display, in which selected key series of slices could be viewed effectively, was found to be suitable for MR image interpretation.

ORIENTATION CHANGE OF CARDIOCYTES INDUCED BY CYCLIC STRETCH STIMULATION

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Mechanical stress has been implicated as one of the growth regulators in the heart. We investigated the effect of cyclic stretch stimulation on morphology and orientation of cultured cardiocytes. Embryonic rat (17-day-p.c.) cardiomyocytes cultured on silicone dishes were cyclically stretched to 120% in length at a frequency of 30 cycles/min. When the cardiocytes were stretched in the initial stage of cultivation (within 12 hr), both cells and intracellular myofibrils oriented parallel to the stretch direction. When the cells were stretched only in the later stage (after 24 hr of cultivation), they tended to orient perpendicular to the stretch. Next we examined the effects of chemical compounds on these phase-related changes in myofibril orientation. None of the drugs tested (H-7, herbimycin A, gadolinium, and EGTA) blocked the parallel orientation induced by the initial stage stretch. By contrast, H-7 and herbimycin A inhibited almost completely the perpendicular orientation induced by the late stage stretch but neither gadolinium nor EGTA did. Our results indicate that the alignment change induced by cyclic stretch depends on the stage of cultivation. The effect of stretch in the later stage is likely mediated by protein kinase C and tyrosine kinase pathways.

ATTENUATION OF REGIONAL DIFFERENTIATION OF SYMPATHETIC NERVE ACTIVITY DURING SLEEP IN HUMANS

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The purpose of the present study is to clarify how the regional differentiation of sympathetic nerve activity is modified during natural sleep in humans. In humans, muscle and skin sympathetic nerve activities [MSNA, SSNA] have been reported to discharge independently during wakefulness according to regional differentiation of sympathetic nerve activity. However, in natural sleep, MSNA and SSNA have been documented to synchronize during sleep stage 2 (Rechtschaffen & Kales). In the present study, we measured MSNA and SSNA simultaneously using a double recording technique of microneurography in eight healthy volunteers during natural sleep, and analyzed how MSNA and SSNA can be synchronized. We found that the synchronicity of MSNA and SSNA was accelerated in correlation with the deepening of the non-rapid eye movement [nonREM] sleep stages. We also documented that the burst properties of MSNA different from those of SSNA in wakefulness become similar to those of SSNA in the nonREM sleep stages, and MSNA synchronizes with SSNA. The synchronicity of MSNA and SSNA is presumably caused by a reduced effect of central inhibitory baroreflex pathways on MSNA during nonREM sleep. The present findings suggest that the regional differentiation

of sympathetic nerve activity is attenuated with the deepening of nonREM sleep stages.

SYMPATHETIC RESPONSES TO CALORIC VESTIBULAR STIMULATION IN HUMANS

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To determine the effect of vestibular stimulation on the sympathetic outflow to muscle and skin in humans, 23 volunteers were examined by irrigation with 50 ml of cold (10°C) or warm (44°C) water into the external meatus. The muscle sympathetic nerve activity (MSNA) from the tibial nerve was recorded in 14 subjects simultaneously with electrooculogram, electrocardiogram, and blood pressure (Finapres). In another 9 volunteers, skin sympathetic nerve activity (SSNA) was measured simultaneously from the tibial and peroneal nerves with the skin blood flow, sweating and electrooculogram. After either cold or warm irrigation, MSNA was enhanced from the latter half of nystagmus and continued after nystagmus. The MSNA increment was proportional to the maximum slow phase velocity (SPV) of the nystagmus. SSNA was suppressed in tibial and peroneal nerves during nystagmus. The degree of SSNA suppression was proportional to the maximum SPV of nystagmus and the duration of SSNA suppression was correlated to the duration of nystagmus. After nystagmus, SSNA was enhanced in some subjects, and the intensity of evoked subjective symptoms of motion sickness was correlated to the increment of SSNA. The results suggest that vestibular stimulation can evoke facilitatory and inhibitory sympathetic nerve responses, eliciting differential sympathetic outflow to different vascular beds.

NEUROPATHOLOGICAL STUDY OF MELAS AUTOPSY CASES WITH SPECIAL REFERENCE TO mtDNA 3243 POINT MUTATION

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Up to now many cases with MELAS have been studied clinically, biochemically, genetically and histopathologically. But neuropathological changes in MELAS have not been studied in detail. Pathological consequence in which abnormal mitochondrial function induces encephalopathy remains unclear. Thus, careful neuropathological examination of MELAS in required to clarify the pathological process in MELAS. We examined the central nervous system of 7 MELAS autopsy cases. Five of 7 cases were confirmed to have mtDNA 3243 point mutation. We here report pathological changes in the cerebellum as well as in cerebrum, mainly in cases with 3243 point mutation. In addition to characteristic multiple necrosis of the cortex, we found

various lesions such as diffuse atrophy of the cortex, diffuse gliosis of the white matter, cactus formation of Purkinje cells and grumose degeneration. These lesions cannot be explained by angiopathy alone, and suggest that other mechanisms including cytopathy contribute to MELAS pathology.

REACTIVITIES OF ANTI-CENTROMERE ANTIBODIES TO THE DIMER FORM OF C-TERMINAL CENTROMERE PROTEIN C

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Centromere protein C (CENP-C), which is one of the centromere autoantigens, can make a dimer at the C-terminus. In order to investigate the reactivities of anti-centromere antibodies (ACA) to the dimer form, the C-terminal 124 amino acids (CF124) was expressed in E.coli and chemically cross-linked into dimers. Immunoblotting was utilized to compare the reactivities between the dimers and the monomers against 58 ACA-positive sera. The reactivities of the dimers were obviously higher than those of the monomers both in IgG and IgM responses. Two kinds of CF124 mutant (each contains one amino acid change at the N-terminal region of CF124) and two cut segments of CF124 (N-terminal 67 amino acids and C-terminal 58 amino acids) were also examined for dimerization activity and antigenicity. Two mutants had less dimerization activity and the C-terminal peptide of CF124 lost it, while the N-terminal peptide could dimerize. ACA did not react to any peptides of the four constructs. These results suggest that there is a conformational epitope derived from a relatively wide region of the C-terminal CENP-C.

THE ROLE OF COMPLEMENT IN THE PATHOGENESIS OF TUBULOINTERSTITIAL LESIONS IN RAT MESANGIAL PROLIFERATIVE GLOMERULONEPHRITIS

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Proteinuria and tubulointerstitial lesions are important signs of progressive renal disease. The purpose of our study was to assess the role of complement in the development of tubulointerstitial lesions in rats with proteinuria due to primary glomerulonephritis. Mesangial proliferative glomerulonephritis was induced in mononephrectomized rats by i.v. injection of mAb1-22-3. As early as 24 h after the injection proteinuria became evident, persisted throughout the observation period, and was associated with mesangial cell proliferation and tub-

ulointerstitial lesions when examined at 7 and 14 days. Deposition of rat C3 and C5b-9 was observed at the luminal surface of proximal tubules (Group I). Rats injected with mAb 1-22-3 and depleted of complement by injections of cobra venom factor starting at Day 3 developed glomerulonephritis and proteinuria comparable to rats of Group I, but complement deposition in the tubules and the tubulointerstitial lesions were markedly reduced (Group II). Rats in Group III were injected with mAb and, from Day 3, with soluble CR1, which became detectable at the luminal surface of proximal tubules and in the urine. Deposition of C5b-9 in tubular cells was not detectable, and tubulointerstitial lesions was reduced compared to rats in Group I. The results indicate that tubulointerstitial lesions is associated with activation of serum complement at the level of tubular brush border, and tubulointerstitial lesions can be reduced by inhibition of complement activity.

EFFECTS OF 17 β-ESTRADIOL AND PROGESTERONE ON MIGRATION OF HUMAN MONOCYTIC THP-1 CELLS STIMULATED BY MINIMALLY OXIDIZED LOW-DENSITY LIPOPROTEIN IN VITRO

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Postmenopausal hormone replacement therapy (HRT) has a beneficial effect on atherosclerotic cardiovascular disease. The aim of this study was to investigate the effects of estrogen and progestin on the migration of monocytes which is one of the important initial steps in the development of atherosclerotic plaque. The presence of estrogen receptors was determined in human monocytic THP-1 cells by Western and Northern blot analysis. While m-ox-LDL increased the migration of THP-1 cells in a dose dependent manner, native LDL had no significant effects on it. Although 17 β-estradiol (E_2) inhibited the m-ox-LDL-induced migration of THP-1 cells in a dose dependent manner, progesterone (P) had no significant effects. The combination of P with E_2 did not show any effect on the inhibitory effect of E_2. Preincubation of THP-1 cells with the anti-estrogenic agent tamoxifen significantly inhibited the effect of E_2. M-ox-LDL stimulated the MCP-1 secretion from THP-1 cells, which was reduced by E_2. Anti human MCP-1 neutralizing antibody inhibited the migration of THP-1 cells stimulated by m-ox-LDL. These findings suggest that the inhibitory effect of E_2 on the migration of monocytes might be one of the factors involved in the decreased incidence of atherosclerotic cardiovascular disease in premenopausal women and postmenopausal HRT.

SEQUENCE DIVERSITY OF SERINE REPEAT ANTIGEN GENE EXON II OF *PLASMODIUM FALCIPARUM* IN WORLDWIDE COLLECTED WILD ISOLATES

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Wild isolates of *Plasmodium falciparum* collected from global endemic areas were analyzed for the genetic diversity of serine repeat antigen (SERA) gene. Two variable regions of SERA exon II in which most of the variation of the gene are accumulated so far as reported in culture strains were amplified by PCR and sequenced. Among 69 isolates examined, deletion/insertion of octamer subunit(s) and non-synonymous subsitutions in octamer repeat region (OR), as well as serine length polymorphism and recombinations in serine repeat region (SR), were revealed to be main mechanisms of variations in the gene. In the OR, all point mutations were non-synonymous, and 16 new allelic forms were found in 40 isolates. But, most of the isolates (66.7%, 46/69) amassed into 3 allelic forms with difference of only 1-2 amino acids. In the SR, 15 variants were detected in 37 isolates. Thus, SERA gene in natural population appears to be neither extremely conserved nor as highly variable as observed in other antigen genes. The allelic forms of Honduras-1 type, one of the three types divided upon reported variations of SR, were likely to be the recombinants of the other two (HB3 and Camp/T9-102) types. Geographically, one group of homologous HB3 variants was found solely in Brazil and another mainly in Solomon Islands. Highest diversity with most of the new allelic forms in both OR (11/16) and SR (11/15) was observed in 21 African isolates. All of three types were detected in Southeast Asis, while no HB3 type and only HB3 variants were detected in African and Brazilian endemic areas, respectively.

MIDKINE IS EXPRESSED DURING REPAIR OF BONE FRACTURE AND PROMOTES CHONDROGENESIS

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Midkine (MK) is a heparin-binding growth/ differentiation factor implicated in control of development and repair of various tissues. Upon fracture of the murine tibia, MK was found to be transiently expressed during bone repair. MK was immunohistochemically detected in spindle-shaped mesenchymal cells at the fracture site on day 4 after fracture, and in chondrocytes in the area of endochondral ossification on day 7. MK expression was decreased on day 14 and scarcely seen on day 28 when bone repair was completed. This mode of MK expression is reminiscent of MK expression during development. MK was expressed in hypertrophyic chondrocytes of the pre-bone cartilage rudiments on embryonic day (E) 14 in

mouse embryos. MK was also strongly expressed in the epiphyseal growth plate. MK was localized intracellularly during both bone repair and development, and this localization was confirmed by immunoelectron microscopy for embryonic chondrocytes. When MK cDNA was transfected into ATDC5 chondrogenic cells and overexpressed, the majority of transfected cells with strong MK expression showed enhanced chondrogenesis as revealed by increased synthesis of sulfated glycosaminoglycans. These results suggested that MK plays important roles in chondrogenesis and contributes to bone formation and repair.

MIDKINE INDUCES HISTAMINE RELEASE FROM MAST CELLS AND THE IMMEDIATE CUTANEOUS RESPONSE

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Midkine is a product of a retinoic acid-responsive gene and exerts a variety of biological activities. The aim of our investigation is to determine whether human midkine have histamine-releasing effects on mast cells, and to show the evidence of the inflammation induced by midkine. Midkine induced histamine release from rat peritoneal mast cells with a rapid response in a dose-dependent manner. Extracellular calcium inhibited the histamine release induced by midkine in a dose-dependent manner. Pertussis toxin and benzalkonium chloride inhibited the histamine release induced by midkine. Gi-proteins exert an effect on the histamine release of midkine. The immediate cutaneous response induced by midkine was positive. These results suggest that midkine may take part in some inflammation via histamine release from mast cells.

MITE ANTIGEN-INDUCED IL-4 AND IL-13 PRODUCTION BY BASOPHILS DERIVED FROM ATOPIC ASTHMA PATIENTS

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There is increasing evidence for the role of basophils in the pathogenesis of atopic diseases such as bronchial asthma. Recently, it has been reported that basophils produce the immunoregulatory cytokines interleukin (IL)-4 and IL-13. In order to investigate the relationship between antigen-specific IgE in the serum and the production of these cytokines and to study how production of these cytokines could be regulated by some anti-asthma drugs, we purified basophils from peripheral venous blood of 67 atopic asthma patients with elevated IgE for the house dust mite. Cells were stimulated with mite antigens for 6 hours and then IL-4 and IL-13 levels in the supernatants were measured by enzyme-linked immunosorbent assay (ELISA). The higher the concentration of mite-specific IgE in the serum, the more IL-4 and IL-13 were pro-

duced by basophils in response to mite antigens. The anti-asthma drugs theophyline and dexamethasone significantly suppressed these cytokine productions. From these results we suggest that antigen-induced IL-4 and IL-13 production by basophils may play an important role in the pathogenesis of atopic asthma. The inhibitory effect of dexamethasone and theophylline on allergic inflammation may be due to the inhibition of IL-4 and IL-13 production not only by T cells but also by basophils.