Department of Cardiovascular Medicine

Outline and Research Objectives

In 1998 the Department of Internal Medicine at University of Tokyo hospital was reorganized to the more functional units based on clinical specialties of diseased organs. Cardiologists from 5 departments of Internal Medicine were unified to the Department of Cardiovascular Medicine. We at quite an early phase introduced techniques of molecular biology into the research field of cardiovascular medicine. As a result, we could report following important findings: 1) phenotypic modulation of cardiac or vascular specific proteins occurs during cardiac hypertrophy or arteriosclerosis. 2) intracellular mechanisms on how mechanical stress causes cardiac hypertrophy; 3) identification of novel genes expressed during heart development; 4) development of animal models for cardiovascular diseases by using genetic engineering techniques (i.e. knockout mice of endothelin-1 and KLF5 genes provided new findings for the research of this field); 5) therapeutic angiogenesis for experimental myocardial infarction by growth factors was originally introduced in this department; 6) We are developing an electronic patient record system by which prognosis as well as risk factors of cardiovascular disease can be statistically analyzed. From a research standpoint, our interests range throughout all fields of cardiovascular medicine ranging from molecular biology to clinical research including genomics. Importantly, our research interests are aimed at making possible new diagnostics and treatment of cardiovascular diseases.

Areas of interest are as follows:

1) Transcriptional regulation in cardiovascular pathogenesis
2) Risk factor analysis of cardiovascular disease
3) Pathogenic mechanisms of cardiac hypertrophy and heart failure
4) Immunological basis of myocarditis and dilated cardiomyopathy
5) MRI in cardiovascular diseases
6) Early diagnosis of ischemic heart disease using radionuclide testing
7) Aerobic threshold and cardiac rehabilitation
8) Anti-arrhythmia therapy using atrial remodeling
9) Mechanism of post-PTCA restenosis
10) Molecular mechanisms of reperfusion injury
11) Genetic polymorphisms in cardiovascular disease
12) Differentiation of smooth muscle cells
13) Cardiac development
14) Gene expression and regulation in cardiomyocytes
15) Mouse genetic models of cardiovascular diseases and vascular development
16) Nitric oxide and endothelial function
17) Gene therapy of heart failure using cardiac contractile proteins
18) Clinical application of vasoactive substances

Faculties and Students

Professor and Chair	Ryozo Nagai, MD, PhD (1999~)
Lecturer	Yasunobu Hirata, MD, PhD
	Minoru Ohno, MD, PhD
	Yoshinori Seko, MD, PhD
Associate	14
Postdoctoral Fellow	11
Graduate student	28
Research student	7
Secretary	4

Past Research and Major Accomplishments

1) Transcriptional regulation of expression of genes related to cardiovascular diseases

We have been focusing our research on the role of transcriptional regulation of gene expression in the cardiovascular system with a particular interest on regulation of phenotypic modulation of smooth muscle and cardiac cells. In 1980s and early 1990s, Nagai et al isolated and characterized three types of smooth

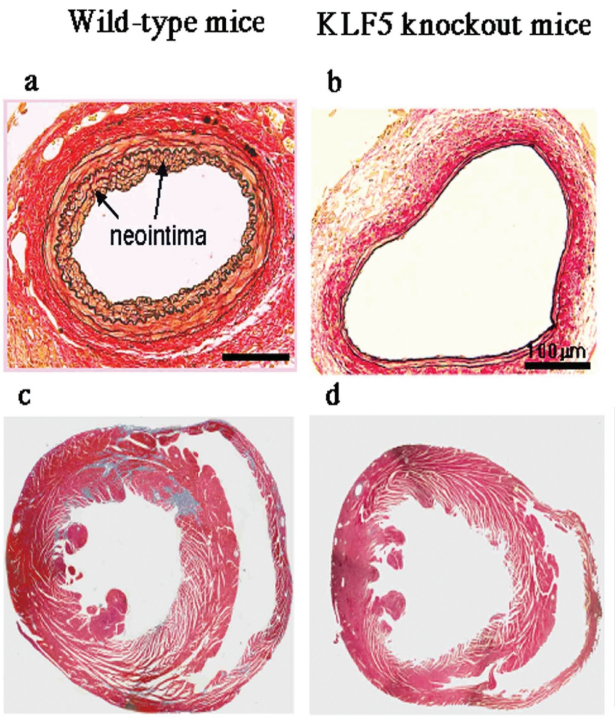

Figure 1

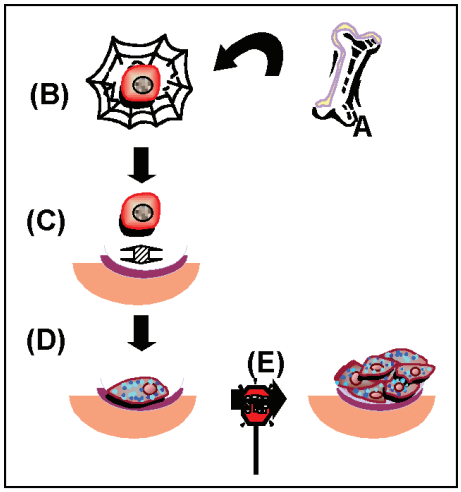

Fig. 2 Strategies targeting vascular progenitors

muscle myosin heavy chains and found that they are differentially expressed during vascular development. Nagai et al furthermore isolated a DNA-binding factor, Krüppel-like factor 5 (KLF5), as a transcription factor of the embryonic isoform of smooth muscle myosin heavy chain gene (SMemb), whose expression is induced in phenotypically modulated smooth muscle cell and cardiac fibroblast. Recently, by developing knockout mice of KLF5 gene, we have found that KLF5 is an essential regulator of cardiovascular remodeling which occurs in response to various external stresses (Figure 1). We have further found that differential chemical modifications and protein-protein interactions regulate this family of factors. We are currently investigating whether low molecular weight compounds that inhibit functions of KLF5 are clinically applicable as a new therapeutic drug against cardiovascular diseases.

2) Differentiation of smooth muscle cells in vascular lesions

On the contrary to general assumption that neointima cells are derived from medial smooth muscle cells, we found that bone marrow cells give rise to the majority of smooth muscle cells that contribute to arterial remodeling in models of post-angioplasty restenosis, graft vasculopathy and hyperlipidemia-induced atherosclerosis. Notably, we found purified hematopoietic stem cells differentiated into smooth muscle cells *in vitro* and *in vivo*. We proposed that among blood cells there may be progenitors of smooth muscle cells, which attach to the injured endothelia, differentiate into smooth muscle cells and proliferate, contributing thereby to neointima formation. Our results will also suggest a novel strategy to prevent vascular diseases, targeting mobilization (A), circulation (B), homing (C), differentiation (D) and proliferation (E) of putative smooth muscle progenitor cells (Figure 2).

3) Establishment of clinical data management system and its practical application to genetic epidemiology in cardiovascular medicine

We have established a clinical data analysis system on network, which enrolls more than 2000 patients who underwent coronary angiography in our department. By using this system we can analyze the relationship between the genetic polymorphisms and the clinical of cardiovascular diseases such as coronary artery diseases (CAD) and ischemic stroke. We demonstrated that the alanine/valine (A/V) polymorphism in the gene of 5,10-methylenetetrahydrofolate reductase (MTHFR) geneis a significant genetic risk factor for CAD and ischemic stroke. This genetic variant is a more powerful predictor of atherosclerotic diseases, when the plasma folate levels are relatively low.

4) Development of mouse genetic models of cardiovascular diseases

Our research interests include the pathophysiological assessment of cardiovascular diseases by mice gene engineering approach. We have generated knockout and transgenic mice of adrenomedullin, ADAMTS-1, endothelin-1 and KLF5. We analyzed the role of these genes and found that their expression is essential for normal growth of mice.

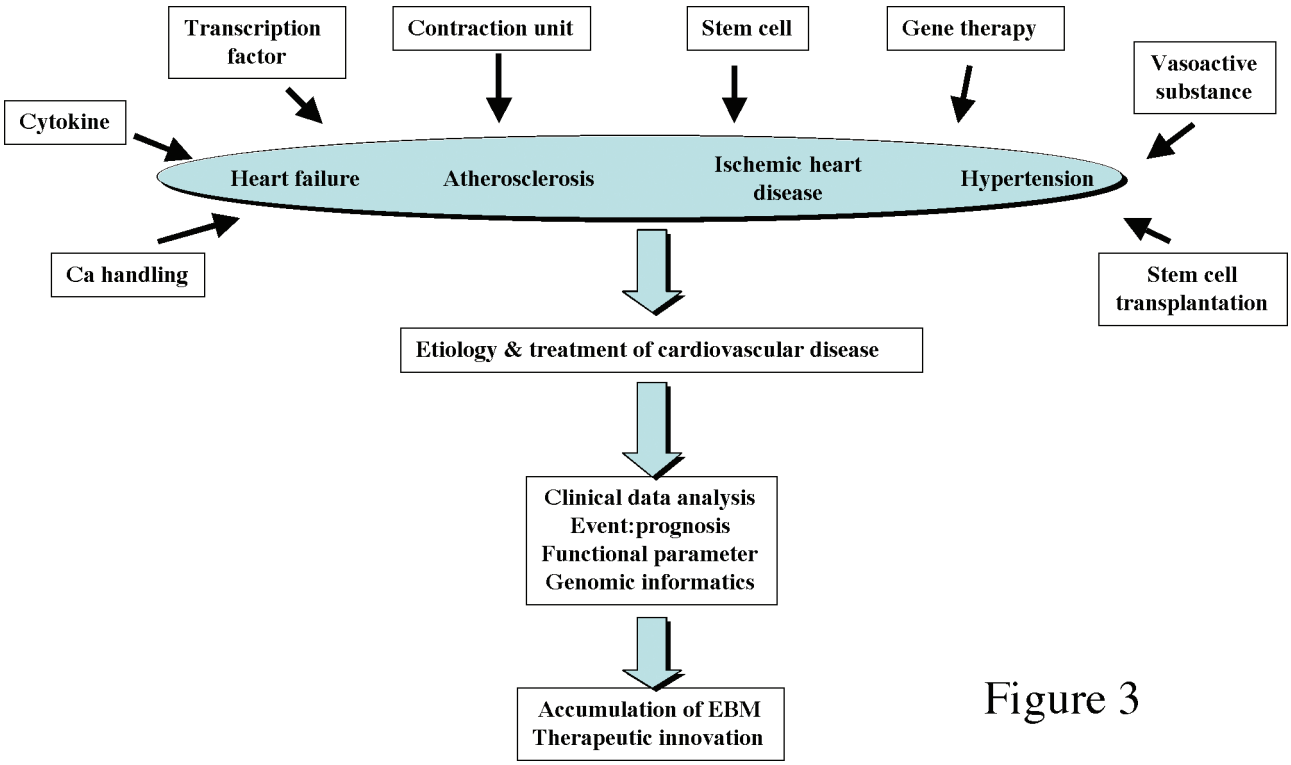

Figure 3 summarizes our strategy for clinical application of basic research on cardiovascular disease.

Current Research

1) Transcription factor such as Krüppel-like factors in pathogenic mechanisms.

Modulation of transcription factors may lead to a new therapeutic strategy for cardiovascular disease. Ongoing studies include using proteomics approaches to understand the diverse protein-protein interactions and regulation of these Krüppel-like factors. We have already identified a novel repressor protein among others. Another focus of research surrounds understanding the transcriptional regulation of these Krüppel-like factors in the context of chromatin which is necessary to understand how transcription occurs in humans.

2) Bone marrow-derived vascular progenitor cells

(1) Identification of bone marrow fraction contributing to atherosclerosis

To determine which fraction of bone marrow cells contribute to the pathogenesis of vascular diseases, we are reconstituting bone marrow of the lethally irradiated recipient mice with the hematopoietic stem cells from LacZ mouse and the mesenchymal stem cells from GFP mouse. We are applying following vascular injury models to bone marrow-reconstitution mice: mouse femoral injury model, heterotopic cardiac transplantation model and atherosclerotic model.

(2) Characterization of bone marrow-derived vascular progenitor cells

By using cell sorter, we are identifying which fraction of peripheral blood cells effectively differentiates into smooth muscle cells. We are determining the cell surface marker to isolate the putative bone marrow-derived smooth muscle progenitor cells. Using bone marrow transplantation mice, we are identifying the effects of hyperlipidemia and drugs on mobilization, homing, differentiation of the progenitor cells in various models of vascular injury described above

3) Establishment of clinical data management system.

To overcome the difficulty of filing complicated clinical parameters for genetic association studies, we are accumulating clinical data and constructing our original database system. For genetic analyses, written informed consent to participate was obtained from all the in-patients in our department. DNA samples obtained from the participants have reached approximately one thousand. Using them, we analyzed over 50 genetic polymorphisms implicated to be associated with atherosclerotic diseases. Among plenty of SNPs analyzed in our study, we showed the polymorphisms in the MMP-1 and MMP-3 promoters are associated with disease susceptibility to myocardial infarction. Also, our database system supports several ongoing clinical epidemiological studies such as J-CAD.

4) Genetic engineering for analysis of the role of endogenous protein

We are analyzing the *in vivo* function of KLF5 using knockout mice. In response to external stress,

KLF5 knockout mice exhibited diminished levels of arterial wall thickening, cardiac hypertrophy, interstitial fibrosis and that angiotensin II induced expression of KLF5, which in turn activated growth factor expression. KLF5 thus appears to be a key element linking external stress and cardiovascular remodeling.

Future Prospects

1) Dissection of the pathogenic mechanisms whereby the Krüppel-like factors are involved in cardiovascular disease will allow us to understand the diverse mechanisms by which this family of factors are involved in transcriptional regulation. By such, we envision that targeted drug design to modulate these factors in this context will enable us to propose a new therapeutic approach to cardiovascular disease (e.g. pin-point drug design).
2) Using our database system we have followed all the cases periodically, thus we can get prospective clinical data such as major cardiovascular events and responsiveness to treatment such as drugs and percutaneous coronary intervention. In future, we will be able to obtain several evidences from our database system and genetic analyses, resulting in clinical application of such evidences in prediction or prevention of major cardiovascular diseases.
3) We have shown that KLF5 is a crucial determinant of the cellular response to cardiovascular injury, playing a key role in mediating cardiovascular remodeling. We are now analyzing the effect of compounds, which could modulate KLF5 function to control remodeling in atherosclerosis and heart failure.
4) Our studies will show that bone marrow cells including hematopoietic stem cells have the potential to give rise to vascular progenitor cells that home in the damaged vessels, differentiate into smooth muscle cells, and proliferate thereby contributing to vascular remodeling. We expect that atherogenic factors such as hyperlipidemia, inflammation, cytokines, and smoking will facilitate the kinetics of smooth muscle progenitors. Our findings will provide the basis for the development of new diagnostic strategies to predict atherosclerosis by quantifying the circulating progenitors.

Research Grants

Ryozo Nagai (1997-2001)
Grant from the Organization for Pharmaceutical Safety and Research
Role of Klotho gene and its clinical application in cardiovascular disease

Ryozo Nagai (1999-2001)
Grant from the Ministry of Health, Labour and Welfare
Research on elucidation of the molecular mechanisms of specific diseases

Ryozo Nagai (2000-2002)
Grant from the Ministry of Education, Culture, Sports, Science and Technology
Development of a new prognostic evaluation system and treatment for patients with cardiovascular disease based on gene polymorphisms

Ryozo Nagai (2000-2002)
Grant from the Ministry of Health, Labor and Welfare
Research on development of angiogenesis and vascular protection treatment

Ryozo Nagai (2002-2006)
Grant from the Ministry of Education, Culture, Sports, Science and Technology
Molecular mechanism and organ remodeling: gene transcription and cell-cell interaction in mesenchymal cells

Select Publications

Diversity of smooth muscle myosin heavy chain isoforms

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Department of Gastroenterology

Department Objectives

The goal of our department is to accurately diagnose and give the best available treatment to patients, and develop better modalities for the future.

To attain the goal, we perform clinical and basic research.

Outline

In 1998, our current Department of Gastroenterology was established through the reorganization of the Divisions of Internal Medicine, and is in charge of busy clinical practices; 5,000 outpatients a month and 1,200 new admissions a year. The most frequent cause of admission is hepatocellular carcinoma, 500 a year, followed by gastrointestinal tract disorders 400, pancreatobiliary 180, and others 120.

Outline-*Hepatology*

Unlike other countries, the majority (more than 90%) of our patients with liver disease are due to *hepatitis virus* infection. Therefore, better understanding of virus replication and pathogenesis may eventually lead to the eradication of the virus and the cure of diseases.

We have developed unique ablation treatment for hepatocellular carcinoma (HCC), for which many patients have visited our department (Fig. 1); we have had 1,600 cases, probably the largest series in the world as a single institute experience.

Outline *-Gastroenterology*

Gastric cancer is the commonest cause of death in Japan. Similar to HCC, there is now enough evidence that bacterial infection, *Helicobacter pylori*, induces or is at least strongly related to cancer development. Along with clinical expertise like *en bloc* resection of cancer (Fig. 2), better understanding of gastric injury due the infection could reduce the most common cause of death in Japan.

Recent increase in the number of colonic cancer is so noticeable. We found that this is mainly due to increase in right sided colon (cecum and ascending colon) cancer in elderly patients. The better understanding of right sided as well as left sided colonic cancers may prevent further increase in the colonic neoplasm in a country where people enjoy unprecedented length of life.

Outline *-Pancreatobiliary*

Pancreatic cancer with incredibly poor prognosis (average survival of 200 days) is insidiously increasing in Japan. There is not even a clue for high risk group. We need, though difficult, to find out high risk groups and better treatment.

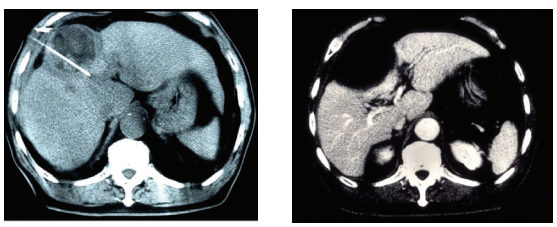

Fig 1. Percutaneous tumor ablation.
(A) During treatment: fine needle was inserted into the tumor.
(B) After treatment: the tumor was completely ablated

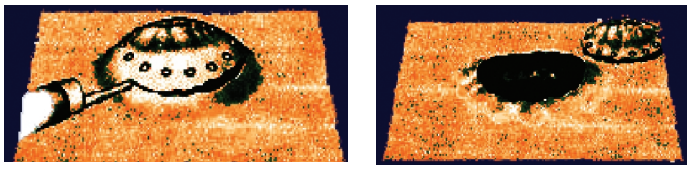

Fig 2. Endoscopic tumor resection.
(A) After injection of saline, the tumor was cut around.
(B) en bloc resection: the tumor was completely removed.

Faculties and Students

Professor and Chair	Masao Omata, M.D., Ph.D. (from 1992)
Lecturer	Takao Kawabe, M.D., Shin Onishi, M.D.

Associate.....19
Postgraduate Fellow 33
Graduate Student.....23
Research Studen 7
Secretary 2

Past Research and Major Accomplishments

1. Acute and Chronic Liver Injury due to HBV (Hepatitis B Virus)

The most severe form of acute liver injury is *fulminant hepatitis* of which 70% is fatal.

However, the underlying mechanisms of severe injury have not been clear.

We elucidated for the first time the fulminant hepatitis was induced by HBV mutant form (N Engl J Med 1991;324:1699-1704).

Furthermore, this mutant carries specific amino acid changes in nucleo-capsid region, a possible target of lymphocyte attack (J Clin Invest 1993;91:1206 - 1213).

These mutations play a critical role not only in acute but also chronic liver disease (J Clin Invest 1992 ;89:332-338, Gastroenterology 1993;104:263-271).

By these understandings, we explored how to treat these wild and mutant strains.

We set up *in vitro* system to maintain these wild and mutant virus replication and identified extremely effective drugs (J Clin Invest1999;103:1635-1640, J Clin Invest 2001;107:449-455).

These drugs are now on clinical trials to prevent fulminant hepatitis and even the decompensated cirrhosis to save patients life.

2. Liver Injury due to HCV (Hepatitis C Virus)

In contrast to HBV, HCV infection is mild but insidious and long-lasting disease to carcinoma.

All of these start with very high chronicity rate (80%) of acute hepatitis. We elucidated for the first time that this is nearly 100% preventable by anti-viral treatment (Lancet 1991;338:914-915, Gastroenterology 1994;107:805-811). Since HCV vaccine has not been developed, the above mentioned is the best available procedure to prevent chronicity. In fact, a study from Germany recently reported identical results (N Engl J Med 2001;345:1452-1457). However, there are stil1 2 million carriers who already have chronic infection. In fact, over 80% of patients with HCC are due to HCV infection in Japan. To prevent HCC, we first revealed natural course of chronic hepatitis to HCC ; this is by step-wise progression of hepatic fibrosis (Ann Intern Med 2000;132:517-524). And eradication of virus by interferon could reduce the incidence of HCC (Ann Intern Med 1999;131:174-181) with resolution of hepatic fibrosis even in cirrhosis, previously thought irreversible (Ann Intern Med 2000;132:517-524). Furthermore, it was recently revealed that overall mortality (not just HCC) was reduced in treatment cohort (Gastroenterology 2002;123: 483-491). By these studies, we now know how to prevent, but question is how to treat patients who already have the cancer. Probably our department is the largest referral center of HCC (500 cases a year), some are from outside Japan, seeking our medical tumor ablation treatment (Fig. 1).

We recently finished our 7-year prospective randomized trial in that we combined radical cure of the cancer nodules by ablation and eradication of HCV by interferon. The 7-year survival of these patients reached 75% (Ann Intern Med 2002; in press). This is compatible or somewhat better than those reported in liver transplantation. When I graduated medical school 30 years ago, liver disease was the one impossible to treat, but now able to envision to how to cure all.

3. Gastric Injury

Gastric cancer is number one cause of death in Japan. More than 80% is supposed to be due to H. pylori infection; 90% eradication of the bacteria is now possible by proper treatment. Thus, we decided to elucidate underlying mechanisms from infection to the cancer, by *in vitro* and *in vivo* studies. First, we tried to identify responsible molecules for inflammation.

In vitro We revealed H. pylori induces strong NF-kB signals, and these are by molecules in Cag pathogenicity island, 40kb segment of 1.5 million nucleotides (Gastroenterology 2000;119:97-108, J Biol Chem 2001;276:44856-44864).

In vivo To extrapolate this to *in vivo* system, we constructed isogenic mutant (knock out bacteria), lacking Cag gene molecules, and infected it to Mongolian gerbils (unique animal model). It turned out among Cag island, Cag E gene is the one responsible for strong induction of inflammation, whereas Vac A, claimed to be inflammation inducing molecule, is not (J Exp Med 2000;192:1601-1609). These CagE molecule of H. pylori gene is generally present in Japanese isolates, whereas it is often absent in European (Gut 1998;42:338-343). These may explain the difference of incidence of the gastric cancer among different countries.

Recently, Kawakami and Tateishi further elucidated that IkB α , a key molecule in NF-kB signal cascade,

was regulated by NEDD8 pathway activating ubiquitin-proteasome degradation system (EMBO J 2001;20:4003-4012, J Cell Biol 2001;155:571-579).

Although the NF-kB signaling is extremely important as an inflammation inducing pathway, mechanism of direct effect on cell proliferation by H. pylori is not known. Hirata recently elucidated that CagA protein strongly activates SRE (Serum Response Element) which might be operating in the formation of polyp and even MALT lymphoma (Gastroenterology 2002; in press).

Our search for *effector protein* to induce signaling cascades has now began (J Exp Med 2000;191:593-602); it may help to eventually elucidate the molecular mechanism of gastric cancer.

4. Colon Cancer

Colonic cancer is catching up with the leading gastric cancer. Shift from Japanese to "Westernized" food may be responsible for this increase. However, we believe there is another reason. We have noticed right sided (cecum and ascending) colon cancer is increasing among patients over age 70 (60% vs 20% in ages over 70 and under 40, respectively) (Gastrointest Endosc 2002;55:548-551). We found the majority of the right sided colon cancer carried microsatellite instability (Cancer Res 1996;56:5620-5623). Now in Japan, average life expectancy for female and male is 85 and 78, respectively, longest ever.

We have initiated genome-wild screening of the genes having dinucleotide repeat or poly-A stretches, targets of repair gene errors.

5. Pancreatic Cancer

Once pancreatic cancer developed, median survival was only 200 days. Although we tried to employ K-ras gene mutation, which is found in 90% of the cancer, for early diagnosis (Gastroenterology 1991;100:233-238, 1996;110:227-231), it has been far from giving benefit to patients.

We have struggled to find better way of treatment and finally decided to apply allogenic stem cell transplantation by collaboration with Department of Hematology and Oncology. So far 5 patients enrolled and results are pending.

Current Research and Clinical Activities

1. Eradication of HBV

As I wrote, the ultimate goal of HBV treatment is not mere suppression which is currently available, but eradication of the virus (Treatment of chronic hepatitis B infection -Editorial N Engl J Med 1998 ; 339:114-115). To attain this goal we are undertaking the following :

HBV has two replication pathways, episomal (N Engl J Med 1986;315:1187-1192) and integrated form into human genome which presumably resist the treatment. We are now intensively focusing our study on the integrated form by use of post-sequencing information on human genome.

2. Eradication of HCV

We could eradicate the virus in more than 90% of acute hepatitis cases as was shown by us (Lancet 1991;338:914-915). However, only 30% in chronic hepatitis. To increase treatment efficacy, we now have *Replicon* system which allows us to grow treatment-resistant strains and to test drug candidates.

3. Liver Injury Mechanisms

Sequencing of extreme small amount of peptides is now feasible by high quality mass spectrometry. We are now analyzing HLA-related wild and mutants virus-derived peptides from human liver. This may identify the target of lymphocyte attack.

4. Prevention and Cure of HCC

We have treated 1,600 cases of HCC so far. We have shown that treatment of virus infection could prevent HCC. However, advanced HCC is still difficult to treat. We found that Vitamin K could efficiently prevent portal tumor invasion, once developed 100% fatal in one year. Along with other modalities, we might expect long-term survival in the majority of the patients with HCC.

5. Gastric Injury Mechanisms

Discovery of H. pylori protein in host cell, phosphorylated CagA protein (J Exp Med 2000;191:593-602), has prompted us to further search the *effector protein*, which could trigger signaling cascades. We have now several candidates.

6. Right-sided Colon Carcinogenesis

As I mentioned, repair-gene system seems deeply involved in the right-sided colon cancer, a prototype is, of course, HNPCC (Hereditary Nonpolyposis Colon Cancer). The understanding may help for people in the country where longest life-expectancy expected.

7. Pancreatic Cancer

We have initiated a large scale genome-wide SNP (Single Nucleotide Polymorphism) analysis to find high risk group.

Others Activites

In 5 years, we presented 121 papers in DDW (Digestive Disease Week, USA), the largest gastroenterology congress.

In 5 years, we published 320 papers in peer review journal.

In 5 years, 52 invited lectures were given in international meetings by one of us (M Omata).

Future Prospective

Education for medical students and for young physicians is the far most important mission of our department for the future. Our department have to cover multiple organs with diverse diseases. Thus, we are trying not to restrict their interest to one specialized field, but rather to introduce general technology and ideas. It will definitely become more "borderless" in post-genome era.

Research Grants

The Organization for Pharmaceutical Safety Research 1997-2001 (Total ¥346,000,000.)

Comprehensive 10-year Strategy for Cancer Control from Ministry of Health and Welfare, Health Sciences Research Grants 1994-1999 (Total ¥100,600,000.)

Medical Frontier Strategy Research from Ministry of Health Labor and Welfare, Health Sciences Research Grants 2001-2003 (2001 / ¥48,000,000.)

Grants-in-aid for Scientific Research on Priority Area from the Ministry of Education, Culture, Sports, Science and Technology of Japan 2000-2003 (Total ¥71,800,000.)

Grants-in-aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan 2001-2004 (Total ¥40,300,000.)

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Department of Nephrology and Endocrinology

Outline and Research Objectives

The Department of Nephrology and Endocrinology is one of the major divisions in the Department of Internal Medicine the University of Tokyo. It covers nephrology, endocrinology, and hypertension. In 1998, the Department of Internal Medicine of the University of Tokyo has been reorganized. Since then, Dr. Fujita has been Professor and Chairman of our department. His achievements touch on hypertension and sodium metabolism. This is one of our main research objectives. The professor and each member of staff have an active responsibility for all research, clinical, and educational activities. There are 12 Assistant Professors supporting the clinical works of our residents in the ward, and also involved in the Hemodialysis Unit. We are working side-by-side in all clinical activities under the supervision of the professor.

In our department, renal biopsy is actively performed and the morphrogical diagnosis of the nephritis is established to give the real benefits of treatment to the patients. We also treat diabetic patients with proteinuria and end-stage renal failure. Every staff physician of our division also works at the hemodialysis unit thus we can manage patients in every stage of renal disease. The endocrine subunit attends a broad spectrum of endocrine entities providing the residents and academic internists a high quality of medical training. It is also our speciality to diagnose and treat secondary hypertension caused by primary aldosteronism, Cushing's syndrome, pheochromocytoma and so on. We often have consultations from the other divisions concerning water and mineral metabolism disorders.

In our division, there are more than 30 students of the Graduate School. This suggests high research activities and outstanding leadership of individual staff in our division. Research seminars are held every Tuesday evening, where both senior investigators and trainees present their own data and experimental plans and discuss profoundly with the professor and all members of our division. In the seminars, scientists outside our department and outside the University of Tokyo including foreign countries sometimes lecture their papers, which encourages our research activities. Moreover, we are actively collaborating with some of the scientists outside our department. Our research topics are covered the almost all field of hypertension, nephrology, and endocrinology.

Faculties and Students

Professor and Chair	Toshiro Fujita. M.D., Ph.D.
Associate Professors	Atsuo Goto, M.D., Ph.D.
	Tomoki Okazaki, M.D., Ph.D.
Lecturers	Shigeo Taniguchi, M.D., Ph.D.
	Kenmei Takaichi, M.D., Ph.D.
Assistant Professors	.....6 nephrologists and 6 endocrinologists
Post-doctorial fellows	9
Graduate Students	38
Research Students	2
Secretaries	2

Past Research and Major Accomplishments

The following major accomplishment has been obtained using the best available molecular, physiological, and morphological techniques as possible.

Vasoactive substances play an important role in not only blood-pressure control but also formation of vascular damages. Thus, we focused on adrenomedullin (AM), a novel vasodilator peptide. To elucidate physiological and pathophysiological function of AM, we generated AM knockout mice. Homozygous AM knockout ($AM^{-/-}$) mice were embryonic lethal but heterozygous ($AM^{+/-}$) mice were grew normally although its AM levels in plasma and tissues were as a half as wild-type mice. Angiotensin II (AII) and salt loading caused severe coronary arterial damages in $AM^{+/-}$ mice. Moreover, $AM^{+/-}$ mice showed enhancement of cuff injury-induced intimal hyperplasia of femoral artery. These changes in $AM^{+/-}$ mice were accompanied with enhanced oxidative stress, suggesting that antioxidant effect of AM is important in its protective effect against vascular damages. Thus, intrinsic AM counteracts AII, and exhibits vasoprotective action.

Insulin resistance has been suggested to play an important role in hypertension and the related organ damages. Thus, we also focused on insulin resistance in several model animals of hypertension. Interestingly, salt loading itself enhanced insulin resistance in normotensive rats. Certainly, salt-induced rise in blood pressure in Dahl salt-sensitive and angiotensin II (AII)-treated rats was accompanied with enhanced insulin resistance. The increased insulin resistance in hypertensive models is intimately related to oxdative stress. Interestingly, aged AM deficient mice showed insulin resistance, associated with overproduction of oxidative stress. Then, this is the model of insulin resistance syndrome, so-called "syndrome X".

Oxidized low density lipoprotein (OxLDL) has been implicated in pathophysiology of atherosclerosis. OxLDL alters endothelial function and triggered atherogenic changes. Thus, we focused on a novel endothelial OxLDL receptor, lectin-like OxLDL receptor-1 (LOX-1). We observed that LOX-1 was enhanced in aortas and kidneys of rats with hypertension, diabetes, and renal failure. Also, LOX-1 was stimulated by oxdative stress, which is known to cause organ damages by common diseases. The expression of LOX-1 was intimately related to nuclear factor (NF)-kB. Thus, LOX-1 was implicated in pathphysiology of atherosclerosis and glomeruloscrelosis via oxidative stress.

To elucidate a novel candidate gene for salt sensitiviy of blood pressure, we performed differential display assay between Dahl salt-sensitive (DS) and –resistant (DR) rats. As a result, we found REPT1 (rat retrovirus-like element transcribed in proximal tubulus) in the kidneys of DS rats. REPT1 was locatd in the proximal tubules and its renal expression was correlated with salt sensitivity among different strains of rats.

Intracellular calcium ion (Ca^{2+}) is a spatially and temporally organized second messenger, which mediates a variety of important functions of vascular endothelial cells ranging from the production of vasoactive substances to gene expression. We developed FRET-based technique to image localized Ca^{2+} signaling in live endothelial cells. And, we succeeded to elucidate localized Ca^{2+} signaling and function of caveolae. Endothelial nitric oxide (NO) was localized in caveolae and activity of NO was regulated by intracellular Ca^{2+} and binding to caveolae.

G proteins control a diverse network of signals. We have focused on mutation of G proteins in human diseases. We have discovered how point mutations in a_s (the a subunits of G_s) alter G_s signaling, causing human diseases. Also, we succeeded in building up a simple cell-free method to study interaction between receptors and G proteins. We have, for the first time, achieved success in designing dominant negative mutant Gα proteins in realistic level. Also, we analyzed relationship of C825T polymorphism of Gβ3 to hypertension, which has been demonstrated in Caucasian people.

We revealed that ionic mechanisms such as activation of inwardly-rectifying potassium currents and activation of nonselective cation currents are important in the regulation of hormone secretion from pituitary cells using electrophysiological techniques. Also, we elucidated the mechanisms of action of regulators of circulation system such as AM, proadrenomedullin N-terminal 20 peptide (PAMP), and AII. In addition, we studied mineralocorticoid action in pathophysiology of cardiovascular diseases.

We studied molecular basis for diseases of calcium (Ca^{2+}) metabolism. For example, we have identified novel mutations in Ca-sensing receptor gene in patients with Type Ⅱ Bartter 's syndrome as well as those with familial hypocalciuric hypercalcemia (FHH). Also, we characterized proteoglycans synthesized by osteoblasts biochemically and explored in detail cell-matrix interactions in osteoblasts. In addition, we identified negative Ca^{2+} responsive element (nCaRE) in the parathyroid hormone (PTH) gene.

From a physiological standpoint, we elucidated the basic transport properties of sodium-bicarbonate (Na^+-HCO_3^-) cotransport (NBC1) in renal proximal tubular acidosis (RTA) with ocular abnormalities. This is an example of a successful translation of basic finding into clinical medicine. We clarified the regulatory mechanism of proximal transport in physiological and pathological states and demonstrated the cAMP-activated chloride (Cl^-) conductance in proximal tubules. We revealed the immunohistochemical localization and function of Kir 7.1 potassium channel, organic anion transporter (OAT1), and stanniocalcin in the rat kidney. We cloned the Na^+/H^+ antiporter (NHE) cDNAs from human tissues and characterized the regulation of the expression of NHE in kidney cells.

With the application to acute renal failure or graft survival in mind, we demonstrated the successful intervention by cyclic RGD (Arg-Gly-Asp) peptide to reduce the renal ischemia-reperfusion model and the diagnistic utilities of monitoring the radio-labbeled RGD peptides. We demonstrated the incorporation of oligonucleotides to proximal tubular epithelial cells and the amelioration of renal ischemia-reperfusion injury using antisense oligonucleotides specificially targeted to inducible NO synthase (NOS). We showed by *in vivo* approach the chemotactic activity of leukotriene B4 to drive neutrophil in acute ischemia-reperfusion injury.

The clarification of the mechanism of the progressive renal disease is the major problem to be solved in the field of nephrology. We contribute much to the

solution of immunological mechanism involved in glomerulonephritis, especially in terms of complement and complement regulatory proteins. We revealed the localization of NOS and NAD(P)H oxidase isoforms in the kidney and gave insight into the functional role of NO and oxidative stress in the kidney of hypertension, glomerulonephritis, and diabetes. We examined the role of physical factors such as mechanical stretch on TGF-β and extracellular matrix accumulation in the progression of renal disease. We demonstrated that renoprotective C-type natriuretic peptide was produced in renal parenchymal cells.

We isolated several transcription factors from vascular myocytes such as an isoform of MEF2A and GATA-6 and identified the implication of these factors in the differentiation or dedifferentiation of vascular myocytes and vascular hypertrophy process. We clarified the intracellular pathway involved in the cell cycle progression with endothelin-1 proved to promote the renal injury.

As a new technique, we developed a simple and reliable method of quantitating mRNAs in minute tissue samples in addition to the established microdissection and single transduction mechanism by which vasopressin and prostaglandins interact in the kidney using immunodissected tubule. We also developed a novel animal model of glomerular endothelial injury, which may help elucidate the mechanism of glomerular endothelial damage.

Research on renal regeneration has just started to do the identification of adult stem cells in the kidney. Using a flow cytometry assay and Hoechst 33342 staining, we isolated adult stem cells in the kidney as side population (SP) cells that have been identified as stem cells in bone marrow, skeletal muscle and liver. Using 3 dimensional culture systems, moreover, cultured renal SP cells differentiate into various kinds of cells.

Troughout our research we have attempted to link our basic finding with our clinical work-----between bench and bedside with either direction.

Current Research

To extent our past accomplishment further, we are now attempting to clarify the following themes.

Recent studies have revealed that oxidative stress is intimately related to not only hypertension but also the other common disease such as diabetes and hypercholesterolemia, so we are examining whether these abnormalities including insulin resistance develop $AM^{+/-}$ mice, in which oxidative stress is enhanced. Also, we are investigating the effect of hypoxia in $AM^{+/-}$ mice, because hypoxia-induced damage is known to relate to oxidative stress.

To elucidate atherogenic role of LOX-1, we are studying whether atherogenic stimuli such as AII infusion and cuff injury cause severe vascular damages in LOX-1 transgenic mice. Moreover, we found that peroxisome proliferator-activated receptor γ (PPARγ) ligands inhibit cytokine-stimulated LOX-1 expression *in vitro* and *in vivo*. Now we are studying this mechanism, which may suggest that the inhibition of LOX-1 might be related to antiatherogenic effect of PPARγ ligands.

We developed FRET-based tool to image subplasmalemmal Ca^{2+} and cAMP. We are now studying role of caveolin scaffolding domain and hsp90 on NO production using unique cell biological and imaging techniques.

By the cell-free method to study interaction between receptors and G proteins, we studied the specificity between many G proteins and G protein-coupled receptors for cardiovascular hormones, such as AII, catecholamines, and AM.

We are studying the central role of Ca-sensing receptor in salt-sensitive hypertensive model animal, in which Ca loading ameliorated hypertension. Moreover, we have many themes such as electrophysiology of pituitary, signal transduction of mineralocorticoid receptor, mechanisms of age-related osteopenia, and so on.

We further clarify molecular mechanism of proximal RTA based on newly identified NBC1 mutation. The molecular basis of ion transport in NBC1 is studying via giant patch technique.

We are studying roles of lipid mediators in renal ischemia-reperfusion injury using several lines of mice deleted/overexpressed lipid mediator genes. We are analyzing detailed intracellular pathways implicated in the expression of monocyte chemoattractant protein-1 in vascular myocytes.

We are investigating role of endogenous vasoactive substances in the progression of renal diseases such as AII, AM, leukotriene, retinoid and glucocorticoids using knockout mice and renal cells. We are also studying roles of oxidative and nitrosoactive stress in the renal diseases including diabetic nephropathy, ischemia-reperfusion injury, and long-term hemodialysis complications.

Now 3 projects of renal regeneration are on going. First, optimizing the 3 dimensional culture systems to differentiate renal SP cells to metanephros. Second, clarifying the condition and identifying the morphogen to differentiate human adult stem cells such as bone marrow derived mesenchymal stem cells into renal components. Third, investigating the existence of stem cell dysfunction in patients with renal dysfunction, and getting comprehensive information for intervention using DNA microarray analysis.

Future Prospects

The main goal of our studies is to cure patients with renal and endocrinological diseases and hypertension to reduce the number of patients with hypertensive complications and renal failure. To serve this purpose, we have to focus our effort to clarify the mechanisms of diseases. There are not only numerous diseases in our field but also multiple mechanisms to involved especially in chronic stage of renal failure and caidiovascular complication-associated hypertension. Thus, we are applying the multi-dimentional approach such as oxidative stress, lipid metabolism, hemodynamics, and vasoactive substabces. These approaches will lead to the development of several new drugs and strategies to treat renal and endocrinological diseases, hypertension, and its complications. By the discoveries of the candidate genes for salt-sensitive hypertension, we can try to choose the suitable antihypertensive therapy and salt restriction for individual patients (tailor-made medicine).

Research Grants

1. Toshiro Fujita: 1998-2002 Grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (specific field): Molecular biological and embryological investigation of adrenomedullin, 54,5000,000 yen
2. Toshiro Fujita: 2000-2002 Grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (B) Role of oxidized LDL/lectin-like oxidized LDL receptor-1 (LOX-1) system in the development of glomerulosclerosis. 15,000,000 yen
3. Atsuo Goto: 1995-1997 Grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (B) Role of endogenous digitalis-like substance in relationship between stress and cardiovascular diseases, 7,500,000 yen
4. Tomoki Okazaki: 1999-2001 Grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (B): The mechanism of transcriptional inhibition by nuclear hormone receptors by using a viamine D receptor as a model, 14,000,000 yen
5. Taro Iiri (Assistant professor of endocrinology): 2002-2004 Grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (A): G protein and diseases: Analysis of molecular mechanism and regulation of signal transduction, 30,000,000 yen

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Department of Allergy and Rheumatology

Outline and Research Objectives

Our department was established in 1998 according to the reorganization of the Internal Medicine. Doctors from previous Department of Medicine and Physical therapy and the Third Department of Internal Medicine have joined our department. Our main clinical field are rheumatology and allergy. Therefore, our research fields also cover the areas including basic immunology, clinical immunology, rheumatology and allergy. Especially, an area called applied immunology such as developing new therapeutic methods has ready became important and some projects in these directions have thus been carried out in our department. Our Reumatology and Immunology field comprises several projects including T cell receptor, autoantibody, oral tolerance, cell signaling and genetic analysis. The allergy field comprises projects of airway inflammation and IgE response.

Faculties and Students

Professor and Chair Kazuhiko Yamamoto, M.D. (Rheumatology, Allergology)1997
Lecturer Hirokazu Okudaira, M.D. (Allergology)
Fujio Takeuchi, M.D. (Rheumatology)
Yosikata Misaki, M.D. (Rheumatology)
Zen-ichiro Honda, M.D.(Rheumatology)
Associate.....8
Postdoctoral Fellow 7
Graduate student 16
Research student.....2
Secretary 4

Past Research and Major Accomplishments

Rheumatology and Immunology

1. T Cell Receptor (TCR) Project

We have established a method for analyzing T-cell clonality using RT-PCR of TCR messages and subsequent electrophoretic separation of the PCR product based on their single strand conformation polymorphism (SSCP). Using this method an analysis of PBLs obtained from a healthy individual revealed the heterogeneity of the CDR3 region of the TCR beta messages. On the other hand, clonally accumulated T cells could be identified as distinct bands on the background heterogeneous electrophoretic patterns. With this method, we reported the importance of antigen-specific T cell clones in the pathogeneses of RA, SLE, other autoimmune diseases.

Having observed the importance of antigen-specific T cells in autoimmune disorders, we are now trying to establish methods to generate regulatory T cells engineered by transfer of genes, the information of which could be obtained from an individual patient sample. At first, we generated an efficient alpha/beta TCR gene transfer system using two independent monocistronic retrovirus vectors. This system enabled us to express the clonotypic TCR in about 25-40% of the CD4+ T cells from murine splenocytes. The transduced cells showed an antigen-specific proliferation in vitro as well as an antigen-specific delayed-type hypersensitivity in vivo. Furthermore, regulatory T cells engineered by the transfer of collagen-specific TCR and IL-10 genes suppressed collagen induced arthritis in mice. In addition, nucloesome-specific T cells generated by the similar triple gene transfer of TCR and CTLA4Ig were applied to lupus-prone NZB/W F1 mice. These cells suppressed the related pathogenic autoantibody production and nephritis without impairing the T cell-dependent humoral immune responses. Thus, from these results, we could obtain efficient TCR function by gene transfer.

2. Autoantibody Project

We have been investigating in the regulatory mechanism of autoantibody production, which is the hallmark of systemic autoimmune diseases such as systemic lupus erythematosus. We generated a transgenic mice which expresses human U1snRNP-A autoantigen. We found that splenic T cells from this transgenic mice suppressed the immune response to human U1-A. In order to further examine the regulation of autoreactive T cell in a clone level, We also generated a transgenic mice expressing ovalbumin (OVA) systemically in nucleus. In the combination with OVA-specific T cell receptor transgenic mice, we

demonstrated that dendritic cells are able to tolerize autoreactive T cells. Furthermore, we found that self-reactive T cells matured into CD4(+)CD25(+) regulatory T cells in the thymus.

3. Oral Tolerance Project

We have studied the mechanism of tolerance induction in the mucosal immune system and its application to therapy of autoimmune diseases. Especially, we focused on the role of DC (dendritic cells) for oral tolerance after antigen feeding. We examined the requirement for tolerance induction in adoptive cell transfer system and found that not only T cells but DCs in Peyer's patches or spleen are important for the transfer of oral tolerance. We also fed OVA to Balb/c mice in bronchial asthma mouse model induced by OVA and confirmed that OVA feeding suppressed airway hypereactivity. In this system, depletion of DCs abrogated the transfer of tolerance.

4. Intracellular Signaling Project

Receptors Fc portion of Immunoglobulines play central roles in the initial step of immune regulation. For the cells to be activated, the receptors should be cross-linked by multivalent ligands. We have engaged in solving the mechanisms, in which physical receptor cross-linking is converted into biochemical signaling. It was assumed that receptor engagement is sensed and converted by Src family kinases (SFKs) to cell-inner events. However, the compensatory function of eight SFKs hampered clear demonstration of the notion. We introduced novel strategies to circumvent the difficulty, and established their requirement and specificity in Fc, and c-Kit and Integrin receptors. Notably, SFKs responsible for Fc receptor signaling are all modified with palmitic acid, and they reside in a membrane domain referred to as 'lipid rafts'.

On the other hand, we found that a 105 kDa moledule was tyrosine phosphorylated through TCR ligation in human T cells. It was proven to be $p105^{CasL}$, a member of the $p130^{Cas}$-related docking protein family, which subsequently binded to the Src homology 2 domain of c-Crk. We showed that it was also tyrosine phosphrylated though integrin stimulation in T cells, and its expression was predominant in lymphocytes. Next, we reported that Fyn and Lck tyrosine kinases regulated tyrosine phosphorylation of $p105^{CasL}$ in TCR-mediated signaling in human T cells and splenocytes of lpr mice, SLE model mice.

5. Genetics Project

We have studied genetic background and pathophysiology of rheumatic diseases, and mechanisms and effect of aging were studied. Examples are

1)Genetic analysis of HLA-DRB1 shared epitope in RA family cases showed the participation of the epitope was neither unique nor indispensable.

2) Linkage analysis using a familial juvenile hyperuicemic nephropathy (FJHN) revealed the susceptibility region in chr16p12.

Allergy

1. Airway Inflammation Project

We established a system to evaluate antigen specific response of the airway in the mouse under a non-invasive, unrestrained condition. Using this system, we analyzed relationships among sensitization to an allergen, increase in airway hyperreactivity (AHR), and development of airway inflammation. The results demonstrated that contrary to the previous general understandings, AHR can be induced with systemic sensitization alone (early phase AHR).

We have also found that intratracheal administration of hepatocyte growth factor (HGF), a regenerative growth factor for bronchial and alveolar epithelial cells could attenuate extent of the experimental lung fibrosis. In addition, we elucidated the mechanism of action and found that HGF has a potent migratory activity and enhancing activity of surface fibrinolytic system of lung epithelial cells. Further, we reported that anti-fibrotic cytokine IFN-γ potentiates the biological activity of HGF by upregulating the expression of c-Met/HGF receptor on alveolar epithelial cells.

2. IgE Response Project

Surface expression of high affinity IgE receptor (FceRI) on mast cells is essential for these cells to exert IgE-dependent effector functions. We have analyzed how and to what extent mast cell surface FceRI expression is regulated, and whether regulation of their surface FceRI levels is functionally important. Through the series of our study, we found that the ligand of FceRI, IgE, which is known to stabilize FceRI, can greatly upregulate the surface FceRI levels on mast cells. A Th2 cytokine, IL-4, is also involved in FceRI upregulation in mast cells. Mast cells with upregulated levels of surface FceRI in the presence of IgE and/or IL-4 demonstrate enhancement in IgE-dependent mediator release and cytokine production. On the other hand, glucocorticoid, decreases the surface FceRI levels of mouse mast cells either in the presence or absence of IgE, via posttranscriptional mechanism. These results indicate that mast cell surface FceRI levels are regulated via multiple mechanisms by various factors including IgE, cytokines and glucocorticoids, resulting in functional alteration of mast cells. IgE- and FceRI-related positive feedback mechanism may account for the pathogenesis of IgE-mediated allergy.

Current Research

Rheumatology and Immunology

1. TCR Project

We are now trying to establish an efficient method to reconstitute TCR function using information available from a small sample of a patient. In order to accomplish these projects, two important technological breakthroughs are necessary. They are cloning techniques of full length cDNA encoding alpha and beta TCR from a single cells and efficient vectors to deliver these genes into usual lymphocytes. We are now trying to develop several possible technical improvements.

2. Autoantibody Project

We have identified several unique molecules to CD4(+)CD25(+) regulatory T cells including transcription factors and proliferation-associated molecules, which indicate that the subset is a unique and distinct population.

3. Oral Tolerance Project

We are studying the mechanisms of generation of regulatory T cells which mediate mucosal tolerance. We are visualizing the interaction between T cells and DCs which captured antigen in Peyer's patch and characterizing DCs which are involved in generation of regulatory T cells. We are also investigating a role of TGF-β for generation of regulatory cells in mucosal tolerance and for class switching of B cells to IgA secreting plasma cells.

4. Intracellular Signaling Project

We are searching for the molecules responsible for Fc receptor-mediated lipid raft assembly through protein chemistry approaches. We have also cloned two novel molecules interacting with a SFK, Lyn. One is an ubiquitin ligase with RING domain, presumably promoting SFK turnover. Another is a huge adaptor protein possessing putative Grb2 and PI3K binding sites and GAP domain.

We are also investigating whether IGF-I could prevent glomerulosclerosis, because glomerulosclerosis was not found in IGF-I overexpressing mice, although glomerulohypertrophy became obvious. To investigate this, we are evaluating the effects of adding IGF-I after adhesion stimulation on some signaling molecules in rat mesangial cells. Concurrently, we are preparing an animal study, using 5/6 nephrectomy as glomeruloscrelosis model rat, to know whether IGF-I infusion prevents glomerulosclerosis.

5. Genetics Project

Some candidate gene analysis are now in progress

Allergy

1. Airway Inflammation Project

We have recently found that passive cell transfer of spleen cells obtained from sensitized mouse into naive mouse provokes an increase in the AHR. This indicates that mechanisms to induce AHR can be studied. We are now analyzing which kind of cell in the lung, such as antigen presenting cells, mast cells, and lymphocytes, would contribute to the development of early phase AHR by negative and positive selection of spleen cells.

2. IgE Response Project

We are interested whether IgE crosslinking stimulation of mast cells and basophils can result in cellular changes other than activation. Currently we are exploring the phenomenon of FceRI-mediated desensitization. At present we think that cellular desensitization and stimulation may be separate but closely related to each other.

Future Prospects

Rheumatology and Immunology

1. TCR Project

Our final goal is to establish an antigen- specific immunotherapy in terms of TCR gene transfer using information from a small sample of an individual patient. Once the basic system is established, we could apply this to several different immunological disorders. Applicable disorders include rheumatoid arthritis, systemic lupus erythematosus and other connective tissue diseases, cancer patients, transplantation, severe infections such as AIDS and severe forms of allergy. We believe that this system would become one of fundamental methods in clinical immunology.

2. Autoantibody Project

We will clarify the generative mechanism of regulatory T cells generation and will innovate the methods to control the regulatory T cells.

3. Oral tolerance Project

Our purpose is to treat autoimmune diseases using the mechanism of tolerance induction by mucosal immune system.

4. Intracellular Signaling Project

Identification of the 'raft target' is unequivocally important for basic science and for therapeutic intervention of inflammatory diseases.

The pathological examination of end-stage renal disease shows similar glomerulosclerotic patterns,

although there are many causes of renal diseases. Therefore, we believe that preventing glomerulosclerosis could stop the progression to end-stage renal disease.

5. Genetics Project

Genes wide screen of several diseases would be expected.

Allergy

1. Airway Inflammation Project

We are expecting to establish a new strategy for preventing the development of AHR before onset of airway inflammation.

2. IgE Response Project

The aim of our study is to understand the pathogenesis and establish the new therapeutic strategy of IgE-mediated allergic diseases, such as bronchial asthma and allergic rhinitis, from which more people are suffering. Because IgE-mediated allergy seems much more complicated than previously thought, our future progress in this research area will be of substantial importance.

Research Grants

1. Research on Specific Diseases from the Ministry of Health and Welfare of Japan 1999-2001.
2. Research on Allergic disease and Immunology from the Ministry of Health, Labour and Welfare 2002-2004
3. Research on Health Sciences focusing on Drug Innovation from the Japan Health Sciences Foundation 2000-2002
4. Research on Eye and Ear Sciences, Immunology, Allergy and Organ Transplantation from the Ministry of Health and Welfare of Japan 1998-2000.
5. Research Grant from the Ministry of Education, Science, Sports and Culture of Japan(Kibann KenkyuA) 1999-2001

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Department of Psychosomatic Medicine

Outline and Research Objectives

Department of Psychosomatic Medicine was founded in 1972 at the Branch Hospital of the University of Tokyo.

Our department started as a clinical section for seeing 'psychosomatic diseases' that are physical diseases whose onset and courses are greatly affected by psychosocial, stress-related factors in the field of internal medicine. However, we have come to cover many patients of eating disorders, panic disorders, and mild depression that are not strictly psychosomatic diseases but share common features of the presence of many physical symptoms and the influence of psychosocial factors.

Based on these historical circumstances, our department has mainly focused on the problems of eating disorders and has also studied panic disorder and mild depression. However, we have also actively studied lifestyle-related and adult diseases such as hypertension (HT), diabetes mellitus (DM), Basedow's disease, and cancers in order to meet the social needs expecting us to see stress-related diseases in the field of internal medicine, investigating what kinds of psychosocial factors are related to their onset and courses and what kinds of psychobehavioral interventions are effective on these diseases. Moreover, we have performed basic and interventional studies of various relaxation procedures that could cancel hazardous effects of stress on our health.

Because psychosomatic medicine on which we rely is rather a new field of medical science born about half a century ago and thus special research methodologies have not been established yet, we should have developed a new method for performing new research. Moreover, the pathological mechanisms of our subjects such as psychosomatic diseases and eating disorders are so complicated by many factors including physical, psychological and behavioral ones, and it has been very difficult for us to empirically uncover their roles one by one.

However, the conditions surrounding our field of research have dramatically changed in recent years, because many new kinds of methods have been developed that can be applied to psychosomatic field including brain functional imaging procedures, ecological momentary assessment (EMA) which means successive monitoring of biopsychobehavioral time series data in daily life, and the discovery and development of basic research of novel eating-related substances. Furthermore, the increase of various psychosocial stress and the acknowledgment of its hazardous effects on health in modern world heighten the social needs for the evidence-based interventional procedures of stress-related diseases far stronger than before. Therefore, our department must greatly contribute to our society in the near future.

Faculties and Students

Professor and Chair	Tomifusa Kuboki, MD,PhD, Since April 1996
Associate Professors	Hiroaki Kumano, MD,PhD
Associate	2
Graduate student	7
Research student	1
Secretary	3

Past Research and Major Accomplishments

We have mainly performed clinical research of patients consulting our department and behavioral scientific research of life-related and adult diseases consulting the department of internal medicine and other physical departments. Furthermore, as a basic research, we have investigated one of the main subjects of psychosomatic medicine that is mind/body correlation meaning interaction between biological, psychological, and behavioral aspects of human being through the study of stress and relaxation. The main fields of our research are as follows and related papers are listed in parentheses the number of which corresponds to the list of Selected Publicatios.

1) Clinical, behavioral scientific, and epidemiological studies on eating disorders (#1,2,3,4,5,10,11,14,16,23,25,30)

2) Behavioral scientific, psychophysiological, and interventional studies on lifestyle-related and adult diseases including DM, HT, Basedow's disease, and cancers (#9,13,17,18,21,22,24,26,28,29,31,33,34)
3) Studies on other clinical subjects of our department including panic disorder and depression (#6,12,20,27,32,36,37)
4) Studies on the development of questionnaires concerning mental health including the TEG, the TSS, the POMS, the LHQ, the SIRI33, and the SSA (#7,28,31,33,35)
5) Psychophysiological and interventional studies on various relaxation procedures such as autogenic training, biofeedback, photic feedback, and Qigong (#8,15,18,19,26,29,34)

Concerning eating disorders, we did the first country-wide epidemiological study and made the mannual of cognitive bahavioral therapy and family guidance for carrying out controlled treatment study collaborating on the projects of Ministry of Health and Welfare. Furthermore, we did many clinical studies for clarifying various features of these patients.

Concerning lifestyle-related and adult diseases, we have introduced advanced methodologies of behaviral sciences and data analysis prodedures including various multivariate analyses. We have gathered many data on how psychobehavioral problems such as lifestyles, stress, and personality could affect the onset and courses of these diseases. These data are useful for clarifying how we should change what kind of behavior for behavior modification that are regarded to be important for the treatment of these diseases.

Stress and mental health are important research themes of psychosomatic medicine, and we investigated hazardous effects of stress by developing various psychobehavioral questionnaires. We got excellent results such as developing the Tokyo University Egogram (TEG) that are now widely used not only in hospitals but also in companies and schools all over Japan, and the Life Health Questinnaire (LHQ) for assessing multiple stress-related variables based on the data of more than 10,000 healthy people. Furthermore, the questionnaire for examining the risk of certain personality trait on the occurrence of cancers named the Short Interpersonal Reactions Inventory (SIRI33) was also developed, and a collaborative study with the National Cancer Center is now in progress.

Because we speculate that the cumulative effects of stress may precipitate or aggravate psychosomatic diseases, panic disorder, or depression, it is urgently necessary to develop therapeutic procedures soothing the effects of stress. We have investigated various relaxation procedures as such stress-reducing methods. As basic research, physical and psychological characteristics of relaxation response have been studied through psychophysioloical measures of autonomic nervous system and electroencephalogram (EEG), and a questionnaire assessing psychological relaxation response has been developed. Furthermore, we have done several interventional studies targeting DM, psyochosomatic diseases, or depression, and excellent results have been obtained such as very positive effects of Qigong group therapy on metabolic control of type 2 DM substantiated by a randomized controlled trial (RCT).

Because psychosomatic medicine is rather a new field of medical science, we should have developed new methods or introduced various methods from adjacent fields for performing new research. As mentioned above, we have achieved several positive results in this point and have made it possible to gather and analyse relevant data, which had never been possible before.

Current Research

We are now advancing more basic research investigating the relationship between physical, psychological and behavioral aspects in the onset and courses of various stress-related diseases using newly developed research methodologies such as noninvasive assessment procedures of brain or autonomic nervous system function and EMA of human time-series bioinformation. Furthermore, it should be noted that targets of research have been expanded to include subjects complaining mainly fatigue who suffer from chronic fatigue syndrome (CFS) and multiple chemical sensitvities (MCS) and both healthy and mildly demented geriatric poplulation.

Now we have four active research laboratories in our department, and each is pursuing various research projects as follows making full use of unique methodologies and collaborating with many outside institutes and researchers.

1) Behavioral Science Laboratory

Objectives: In order to clarify the disease processes and ways of treatment of lifestyle-related diseases and psychosomatic diseases, we should deal with the functional aspect of subjects fucusing on behavior meaning how they live a daily life as well as the anatomical aspect focusing on body and the introspective aspect focusing on mind. The objectives of this laboratory are to investigate behavior as well as its relation with mind and body of various subjects ranging from psychosomatic patients to healty persons.

Methodologies: Using questionnaires, personal degital assistants, psychophysiological assessment

procedures, or blood chemistry, the multiple data concerning behavior, mind and body are gathered. Then, the structure within the data is precisely extracted by making full use of many kinds of data analysis methods such as multivariate analyses, time-series analyses, and analysis of variance.

Projects:

DM: Cross-sectional and longitudinal studies to clarify multiple psychosocial factors affecting metabolic control and quality of life (QOL) of diabetic patients

Tension-type headache: An interventional study by autogenic training

Cancer: A semi-prospective study of the role of type C personality on the onset of prostate cancer; A prospective study of personality factors affecting the onset of various cancers in healthy persons

Renal failure: A cross-sectional study on the relationship between stress-related factors and metabolic control and QOL of hemodialysis patients

MCS: A cross-sectional study on stress-related factors affecting their onset and courses

Depression: To determine the diagnostic criteria of mild depression

Elderly people: Cross-sectional and longitudinal studies to clarify the relationship between bodily function, exercise ability, lifestyle, and stress-related factors

2) Bio-signal Analysis Laboratory

Objectives: Although human body produces various kinds of signals, most of them are not captured and discarded as noise. However, as many novel signal analysis methods have been developed these days, we can now extract valuable information from signals previously regarded as noise and can investigate such a field as has been difficult to be targeted by scientific inquiries. The objectives of this laboratory are to understand pathophysiology, mind/body correlation, stress, or relaxation by using such novel methodologies.

Methodologies: Higher brain functions are analyzed by use of positron emission tomography (PET), functional magnetic resonance imaging (fMRI), magnetic encephalography (MEG), and EEG. Autonomic as well as central nervous functions are estimated by spectral and fractal analyses of heart rate variabilities. Ecological changes of heart rate variabilities, bodily movement, mood, symptoms, and toxic substances in the environment are successively monitored by EMA methods.

Projects:

PET: Measurement of cerebral blood flow or glucose metabolism before and after the treatment of anorexia nervosa or panic disorders; Investigation on the relationship between stress coping processes and cerebral blood flow

EEG: Studies assessing the improvement of cerebral cortical function by some relaxation procedures using the dipole tracing method; Assessment of patients with various diseases by event-related potentials (ERP)

Heart rate variabilities & EMA: A study on autonomic nervous activities and bodily movement in the refeeding phase of anorexia nervosa; Development of autonomic nervous function database of outpatients in our department; Comprehensive assessment of multiple chemical sensitivities and chronic fatigue syndrome by EMA methods

3) Neuro-immuno-endocrinology Laboratory

Objectives: The biological basis of mind/body correlation is regarded as 'functional association between neuroendocrine and immune systems', that is sought to be clarified by clinical as well as basic methods in this laboratory. Brain mechanism affecting abnormal eating behavior is also a main focus to be inquired. In addition, the search for physiological measures of stress responses and their application to clinical field is another challenge.

Methodologies: Methods of functional morphology and behavioral analysis are used for analyzing genetically-modified mice such as a knockout mouse. We take notice of 8-hydroxy-2'-deoxy guanosine (8OHdG) as an oxidated substance of DNA and measure it in human urine.

Projects:

Inflammatory cytokines: Analyses of expression and mechanism of action in brain tissue of inflammatory cytokines such as IL-1 and IL-6 as modulators of neuroendocrine and immune systems

Eating modulating substances: Investigation on the effects of newly-found neuropeptides such as orexin and ghrelin in brain on emotion and stress responses

Novel stress measure: Investigation on the usefulness of 8OHdG as a physiological measure in human stress-related diseases

4) Eating Disorders Laboratory

Objectives: The etiology of eating disorders such as anorexia nervosa and bulimia nervosa was assumed to be affected by many factors including individual psychological, biological, familial, socio-cultural factors. The objectives of this laboratory are to study various factors relating to the courses of eating disorders, to develop and improve treatment procedures, and to do the basic research of pathophysiology collaborating with other laboratories.

Methodologies: Bio-psycho-behavioral clinical data at the initial visit and during treatment courses are gathered; Pathophysiology of eating disorders is investigated by PET or EMA.

Projects:

Dropout: A study by survival analysis on premature termination of outpatient treatment

Refeeding: Assessment of energy metabolism, autonomic nervous function, and bodily movement in the refeeding phase of anorexia nervosa patients

Cerebral blood flow and dopaminergic metabolism: Pet studies of anorexia nervosa patients

Future Prospects

We want to continue to advance activities of above-mentioned four laboratories and to focus on the following points in particular.

1) Brain processes closely related to mind/body correlation in various stress-related diseases and pathogenic brain functional abnormalities will be clarified by active use of functional brain analysis methods such as PET, fMRI, MEG, ERP, and dipole tracing methods.
2) Ecological changes in everyday life of biological, psychological, behavioral, and environmental measures will be monitored and analyzed in order to characterize neurobehavioral basis of various stress-related diseases by developing and utilizing relevant EMA methods.
3) Treatment programs will be developed that intervene psychobehavioral factors found to be closely related to the processes of psychosomatic diseases and lifestyle-related diseases by our behavioral scientific studies and include effective relaxation procedures. Then, the evidence will be shown that these interventions are effective in improving not only symptoms but also QOL and life satisfaction by carrying out RCT of these treatment programs.
4) Biological and material basis of eating disorders will be pursued by both basic research such as one using genetically-modified mice and clinical research measuring eating-related substances in the blood of eating disorders patients before and after oral glucose tolerance tests.

In summary, we are going to advance toward two directions. First, we will change over from the previous research system mainly relying on clinical and psychobehavioral domains to the new research system that enables us to investigate neurobehavioral and biological mechanisms actively utilizing novel methodologies. Then, we will accumulate useful data for the development of biological treatment by broadening our horizons from mind/body correlation to mind/brain/body correlation and by seeking for the true nature of pathological processes of stress-related diseases.

Secondly, we will make it possible to gather diverse data in patients' everyday life by incorporating EMA into behavioral scientific methodologies, and then we will aim to clarify the reality of not only diseases themselves but also patients having such diseases. Based on these results as well as our present knowledge, we will develop treatment programs including interventions manipulating psychobehavioral variables and relaxation procedures and will clarify by RCT what kinds of interventions most effectively improve not only symptoms but also QOL and life satisfaction.

Research Grant

1) Grant of Ministry of Health, Labor and Welfare Sick House Syndrome(SHS)---Studies of patho-physiology, method of diagnosis and its treatment.
2) Grant of Ministry of Health, Labor and Welfare Eating disorder- Studies of neurology and endocrinology.
3) Grant of Ministry of Health, Labor and Welfare A guideline for the diagnosis and treatment of psychosomatic diseases 2002
4) Grant of Ministry of Health and Welfare Studies of stress management

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Department of Clinical Laboratory Medicine

Outline and Research Objectives

Department of Clinical Laboratory Medicine, Tokyo University Graduate School of Medicine, was established in 1997 following foundation of Department of Laboratory Medicine, Faculty of Medicine, University of Tokyo in 1984. We have also Clinical Laboratory Center which was established in 1952 in Tokyo University Hospital. The Department cooperates with Clinical Laboratory Center not only in giving lectures and clinical education to medical students but also in carrying out researches. The Department of Clinical Laboratory Medicine is composed of only two staffs, a professor and an associate professor, but Clinical Laboratory Center possesses four positions of lecturers and two of associates. The professor and the associate professor of the Department are the director and the vice-director of Clinical Laboratory Center, respectively.

In our department five or six research projects have been usually undertaken.

Faculties and Students

Professor and Chair Kazuhiko Nakahara, M.D. (1997~)
Associate Professor Kiyoshi Kitamura, M.D.(1998~2002)
Graduate Student.....1
Secretary 1

Past Research and Major Accomplishments

1. Cell surface analysis using flow cytometry

The major purpose of this project was to apply the immunological methods to actual clinical fields, for instance to diagnosis hematological malignancies such as leukemia and lymphoma. We found that a kind of glycolipids, asialo GM1, is the candidate of cell surface markers for diagnosis of acute lymphoblastic leukemia by using the method of flow cytometry. For the next steps in this project, we discovered flow cytometric analysis using several monoclonal antibodies reacted against human blood cells, especially lymphocytes, is very useful tool to diagnose human hematological disorders. To give an example, we mentioned that adult T-cell leukemia cells are CD4 positive but negative for CD8, and very clear pattern of the positive sharp peak could be shown on the display of flow cytometry. We also demonstrated that not only single-color but also multi-color analysis with monoclonal antibodies and flow cytometry is very important to investigate some kinds of illness in more detail.

For the purpose of evaluating lymphocyte activation, we have studied lymphocyte surface antigens, plasma soluble CD4 or CD8, and intracellular pH (pHi) of lymphocytes, especially in infectious mononucleosis (IM) in which proliferation of T lymphocytes is supposed to play an important role in regulation of EB virus. Immunofluorescence analysis demonstrates increase or decrease of lymphocyte subset as well as expression of activation antigens. During IM, $CD8^+$ T cells are quantitatively the major population of expanded lymphocytes and they coexpress HLA-DR and CD45RO. Elevated serum levels of soluble CD8 (sCD8) and soluble CD4 (sCD4) are regarded as markers of T cell activation. We demonstrated marked increase in sCD8 and significant increase in sCD4 which strongly suggest activation of both $CD8^+$ and $CD4^+$. The elevation of plasma sCD8 is due to expansion of $CD8^+$ subset as well as increased sCD8 release from each $CD8^+$ cell.

Additionally we tried to apply the flow-cytometric analysis method to measure intracellular pH (pHi), which shows one of the indices of cellular activation. For the purpose of evaluating intracellular pH of each lymphocyte subset, simultaneous measurement of pHi and cell surface antigen was developed using 2'-7'-bis(carboxyethyl)5,6-carboxyfluorescein (BCECF) and flow-cytometry. A significant increase in pHi of $CD8^+$ lymphocytes from patients with IM and its correlation with number of $CD8^+$-HLA-DR^+ lymphocytes as well as plasma levels of soluble CD8 strongly suggest activation of $CD8^+$ lymphocytes.

2. Effects of age, body-mass index and life-style habits on clinical laboratory data.

2-1 Alcohol drinking and clinical laboratory data

Epidemiological studies have consistently shown that light or light/moderate drinkers are at a lower risk of coronary heart disease (CHD). The mecha-

nisms of this association include beneficial effects on HDL- and LDL-cholesterol, insulin sensitivity, platelet aggregation, blood coagulation and fibrinolysis. However, drinking also has disadvantageous effects on blood pressure, triglycerides and uric acid.

The purpose of our study was 1) to clarify whether there are individual varieties regarding the response of clinical laboratory data to alcohol consumption, and 2) what are the factors responsible for the individual varieties.

2-1-1 Are there individual varieties regarding the response of clinical laboratory data to alcohol consumption?

We performed a cross-sectional study of 3130 Japanese male workers with a body-mass index below 24. The subjects were divided into 2 groups; a normal r-glutamyl transpeptidase (rGTP) (<40 IU/L) group and a high rGTP (>40 IU/L) group, and the values were compared after adjusted for age, body-mass index, exercise and smoking. The level of triglycerides increased according to the amount of drinking in the high rGTP group, whereas no association was observed in the normal rGTP group. The level of HDL-cholesterol increased with drinking in the normal and high rGTP groups, and no difference was observed in the levels of HDL-cholesterol between the two groups. The levels of uric acid and blood pressure also increased with drinking in both groups, but the increase was bigger in the high rGTP group than in the normal rGTP group. The results indicated that there was large individual variability in the responses of the risk factors for coronary heart disease to drinking. Subjects whose rGTP responds less to drinking may have less disadvantageous effects of drinking.

2-1-2 Is there any good way of drinking for health?

We performed a cross-sectional study of 3660 male workers to examine whether the weekly frequency of drinking affects clinical laboratory data when the weekly amount of drinking is the same. The results suggested that the weekly frequency of drinking might affect the levels of HDL-cholesterol independently of the weekly alcohol consumption.

2-1-3 Are there the genetic factors responsible for individual varieties regarding the response of clinical laboratory data to alcohol consumption?

We examined 133 male workers who drank > 300g of alcohol per week to sudy the relationship between clinical laboratory data and the genetic polymorphisms of alcohol dehydrogenase (ADH) 2 and aldehyde dehydrogenase (ALDH) 2. The results suggested that the ADH2 genotype influences the responses of blood pressure, triglycerides, and uric acid to alcohol consumption, while the ALDH2 genotype influences the response of the erythrocyte mean cell volume to alcohol consumption.

2-2 Others (HbA1C, proteinuria, leptin)

2-2-1 HbA1C

HbA1C has been suggested to deteriorate with age. However, controversy exists as to whether this deterioration is a consequence of the aging process itself or of variables related to aging, such as concomitant disease, medication, body-mass index, physical activity, and the change in distribution of body fat. We performed a cross-sectional survey of 7664 male Japanese workers aged 20-59 years. This survey suggested that HbA1C increased with age itself, and body-mass index and a hereditary predisposition to diabetes affected this age-dependent increase in HbA1C.

2-2-2 proteinuria

We performed a cross-sectional survey of 5174 male Japanese workers. Blood pressure and a family history of diabetes were independent factors associated with proteinuria in subjects with a HbA1C below 5.9% who were not under medication for diabetes. In contrast, HbA1C, obesity and smoking were associated with proteinuria in subjects who were under medication for diabetes and/or have a HbA1C above 5.9%. These findings suggest that maintaining a HbA1C level below 5.9%, non-smoking and a standard body weight may reduce the prevalence of proteinuria in Japanese men. Healthy life–style habits and standard body weight are especially important for subjects with a family history of diabetes.

We also performed a cross-sectional study of 237 men to examine the relationship between proteinuria determined with a reagent strip and the angiotensin converting enzyme (ACE) genotypes. The results suggest that the ACE DD genotype may be an independent risk factor of proteinuria in Japanese men.

2-2-3 lepein

Leptin, the ob gene product secreted by adipocyte, decreases food intake while it increases energy expenditure and functions as an important signal for the regulation of body weight in mouse. To investigate whether smoking affects the serum level of leptin, we performed a cross-sectional study of 708 male workers. The findings suggested that among life-style habits, physical activity, but not smoking or alcohol consumption, significantly affects the serum level of leptin in Japanese men.

The leptin receptor is an isoform of the B219 gene product, a member of the hematopoietin receptor family, which is expressed in very primitive hematopoietic cells. Recent studies showed that leptin plus erythropoietin acted synergistically to increase

erythroid development in vitro. These finding led us to examine the relationship between the serum levels of leptin and hemoglobin. The results of the above cross-sectional study showed that the serum levels of leptin is negatively associated with those of hemoglobin. Although the effect of leptin on hematopoiesis may be modest, the results of our epidemiologic study, together with the previous studies performed in vitro, suggest that leptin may play some role in hematopoiesis in humans.

3. Learning from patients at the points of biochemistry

3-1 Study of apolipoprotein A-1 deficiency

We evaluated a 69-year-old Japanese woman with apolipoprotein (apo) A-I deficiency, high levels of LDL-cholesterol, hypertension and impaired glucose tolerance. The patient had corneal opacity, but neither xanthomas, xanthelasma, nor tonsillar hypertrophy. She was not symptomatic for coronary heart disease, and had normal electrocardiograms at rest and during exercise on a cycle ergometer. She had severely reduced levels of HDL-cholesterol (0.10-0.18 mmol/L) and no apo A-I (<0.6 mg/dL). LDL-cholesterol and apo B as well as apo E were increased even under treatment with 10 mg pravastatin per day. Gel filtration chromatography revealed that in addition to VLDL and LDL fractions, she had apo A-II rich and apo E rich fractions, which were present in the HDL fraction separated by ultracentrifugation. A cytosine deletion was identified by genomic DNA sequencing of the apo A-I gene of the patient at the third base of codon 184 in the fourth exon, which led to a frame shift mutation and early termination at codon 200. This patient is the oldest among those with apo A-I deficiency reported in the literature, and she had no symptoms of coronary heart disease despite the accumulated risk for the disease.

3-2 Study of phytosterolemia

We diagnosed two women with multiple xanthomas, intermittent arthritis and thrombocytopenia as phytosterolemia, an autosomal-recessive lipid storage disease, based on their increased serum concentrations of β-sitosterl, campesterol and sitostanol. The gene responsible for this disease is located within a distance of 18 cM between microssatellite markers of D2S1788 and D2S1352 at chromosome 2p21. We genotyped the patients and their family members with 16 microsatellite markers around this locus. The results from the homozygosity mapping of one family suggested that the gene was located within the distance of 12.6 cM between D2S2328 and D2S1352. We have shortened the genetic distance by 5.4 cM.

3-3 Another good effect of pioglitazone, an insulin sensitizing drug

The findings obtained from two patients with diabetes and asthma suggested that pioglitazone may ameliorate symptoms of asthma. Pioglitazone is one of the thiazolidinedione compounds which have been used as antidiabetic drugs. Recent studies have revealed that thiazolidinedione compounds also have various nonhypoglycemic effects, such as antiinflamatory, antiatherosclerotic and anticancer effects, in cultured cells and in experimental animal models. Elucidation of the mechanism may enable the design of a novel class of drugs for asthma.

4. Researches in physiological fields

4-1 Cardiac functions using ultrasound

Major research interests are evaluation of left ventricular and left atrial functions, detection of tissue ischemia using contrast echo, and assessment of cardiac state during inactivity.

4-2 Influence of diesel exhaust particles on respiratory functions

We reported that diesel exhaust particles (DEP) induced nuclear factor-kappa B(NF κ B) activation, upregulated expression of intercellular adhesion molecule(ICAM)-1 in human bronchial epithelial cells, and benzene-extracted components are important for the major activity of DEP. Increased expression of transforming growth factor-β 1 in small airway epithelium was also demonstrated.

4-3 Investigations on brain functions using magnetoencephalography (MEG)

We installed the MEG system, which is a newly developed tool for neurophysiology, and studied somatosensory evoked magnetic fields to on- and offsets of rectangular pressure stimuli.

Current Research

1. Quantification of cell surface antigens using flow cytometry

Conventional methods of flow cytometry provide us only a positive percentage of cells. If we can measure and obtain cell surface antigen density, more detail analysis must be possible. The purpose of this project is quantification of cell surface antigens with flow cytometry.

2. Analysis of Th1/Th2, Tc1/Tc2 lymphocytes using flow cytometry

A method to analyze blood samples with EDTA-2K

was developed. Infectious diseases and immunocompromised patients are under investigation.

3. Relationship between genetic polymorphisms of alcohol metabolizing enzymes, and diabetic control and the risk factors for CHD in patients with diabetes.

Subjects: Non-drinkers and drinkers with diabetes

Methods: Genetic polymorphisms of ADH2 and ALDH2 are examined by the PCR.

Objective:

1) Is the effect of alcohol drinking on diabetic control associated with the genetic polymorphisms of ADH2 and ALDH2?
2) Is the effect of alcohol drinking on the risk factors for CHD associated with the genetic polymorphisms of ADH2 and ALDH2?
3) Are those effects observed only in drinkers and not in non-drinkers?

4. Identification of the gene mutation responsible for phytosterolemia in our three patients.

Recent studies showed that phytosterolemia might be explained by mutations of the genes that encode new members of the ATP-binding cassette (ABC) transporter family, ABCG5 and ABCG8. Now we are examining whether our patients have the gene mutations of ABCG5 and ABCG8.

5. Physiological roles of FGF-23

A new comer to our Department has cloned and identified FGF-23 as a causative factors for tumor-induced hypophosphatemic rickets/osteomalacia. We have also established an ELISA assay for biologically active full-length FGF-23. We are now investigating physiological roles of FGF-23 in phosphate and vitamin D metabolism.

6. Adrenomodullin

As another new comer participated in our Department, this new project is started. He has been studying the physiological role of vasoactive peptide to regulate blood pressure by modulating sympathetic tone and its possible role in preventing organ damage. Adrenomedullin, a vasoactive peptide was discovered from pheochromocytoma cells and it is known to exist not only adrenal cells but also vascular smooth muscle cells, endothelial cells, and other organs. He and his colleagues have generated adrenomedullin knockout mice and showed that adrenomedullin prevents organ damage by reducing oxidative stress. In clinical study, they have shown adrenomedullin is better indicator of circulating blood volume than ANP in patients under hemodialysis. The measurement of blood adrenomedullin level will be clinically important marker. On the other hand, to diagnose organ damage, especially renal damage, we are now investigating new marker in urine from various patients.

7. Analysis of perfusion and function of the left ventricule

Myocardial perfusion is assessed by myocardial contrast echo while regional left ventricular function is evaluated by strain data derived from myocardial Doppler velocity information.

8. Relationship between respiratory function and various pathophysiological condition

The influence of sleep apnea syndrome, lung cancer, asthma, aging or respiratory rehabilitation on pulmonary function has been investigated by a member of our Department.

9. Study using magnetoencephalography (MEG)

We have not only been making efforts to obtain reimbursement for clinical MEG studies but also been organizing several research groups which consists of medical doctors from the department of neurology, physiology, neurosurgery, pediatrics and otolaryngology in the University Hospital and researchers from University of Tokyo School of Medicine. Current themes are follows.

1) Epilepsy evaluation-spike source localization
2) Pre-surgical functional mapping-somatosensory, motor, auditory, visual and language
3) Auditory evoked magnetic response as a measure of sensory gating in schizophrenia
4) Abnormality of mismatch negativity in schizophrenic patients

Promote

Future Prospects

We have a future planning in each research theme as follows.

1) Apply the quantity analysis method of cell surface antigens with flow cytometry to actual clinical samples and evaluate its usefulness.
2) Monitor immunological status of immunocompromised patients and patients with infectious diseases by using lymphocyte activation markers.
3) Prospective study on the relation between the mortality due to CHD and alcohol drinking, which takes genetic polymorphisms of alcohol metabolizing enzymes into consideration
4) Elucidation of the structures and functions of ABCG5 and ABCG8 and design of a novel class of drugs for hypercholesterolemia
5) Study on physiological and pathophysiologocal roles of FGF-23

6) Find new markers to diagnose early phase of renal damage in diabetes or hypertensive patients as well as to diagnose response to treatment.
7) Three dimensional analysis of left ventricular perfusion and function using cardiac ultrasonography
8) Promote EEG and MEG studies of higher cognitive brain function, especially focused on the investigation of gamma-band activities which are thought to play a key roll in perception and cognition.

It might be said that the research themes of our department are diverse and contain a wide range of subjects. Although we have actually a lot of research projects simultaneously, we can say that it is the characteristic of our department and our common theme is laboratory examination and the key word is laboratory medicine. The purpose of our research is to apply our research products to actual clinical fields, and contribute to human health and welfare. But today's problem in our department is the shortage of the number of graduate students. We must make an effort to obtain them.

Research Grants

1. Grant-in-Aid for Scientific Research (B) from The Ministry of Education, Science, Sports and Culture (1998~2000) : The new application of flow cytometry – Quantification of cell surface antigens and its clinical application –
2. Grant-in-Aid for Scientific Research (B) from The Ministry of Education, Science, Sports and Culture (2001~2003) : Practical utilization of selective cell information using flow cytometry and its clinical application
3. Grant-in-Aid for Scientific Research (C) from The Ministry of Education, Science, Sports and Culture(1999/2000) : Studies on phytosterolemia: Identification of the pathologic gene and examination of its pathogenesis
4. Grant-in-Aid for Scientific Research (C) from The Ministry of Education, Science, Sports and Culture (2001/2002) : Reverse cholesterol transport in apolipoprotein A1 deficiency
5. Grants-in-aid for Promotion of AIDS Research from the Ministry of Health and Welfare of Japan (2000~2002)

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1. Nakahara, K., Ohashi, T., Oda, T., Hirano, T., Kasai, M., Okumura, K.& Tada, T. : Asialo GM 1 as a cell-surface marker detected in acute lymphoblastic leukemia. New England J Medicine. 302 : 674-677, 1980.
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Department of Transfusion Medicine

Outline and Research Objectives

The Department of Transfusion Medicine was established in 1949, and actually is composed by 4 medical doctors, 10 medical technicians, 1 nurse and 1 office assistant.

Faculties and Students

Professor and Chair	Yoichi Shibata, M.D., Ph.D. (since April 1993)	
Associate Professors	absent	
Lecturer	absent	
Associate	3	
Research Students	4	research students from the department of Surgical Oncology are developing their research projects at the department's laboratory.
Secretary	1	
Medical Technician	10	
Nurse	1	

Past Research and Major Accomplishments

The major research projects developed at the department are related to:

1) Human platelet antigen/antibody detection and characterization: the mixed-passive hemagglutination (MPHA) assay, a serological method largely used in Japan for the screening and characterization of platelet alloantigens and alloantibodies, was developed at the department by Yoichi Shibata. Using this method, a new platelet alloantigen system, namely the HPA-4 system (Yuka/b), was discovered by Y. Shibata, et al.
2) Human endothelial cell antigen/antibody detection and characterization: the MPHA method, developed by Y. Shibata, et al., was modified for the detection and characterization of endothelial cell alloantigens and alloantibodies. Using this method, monoclonal antibodies reactive with tumor endothelial cells were developed at the department. This monoclonals are being investigated for a possible application in cancer treatment.
3) Immunotherapy of cancer using LAK cells and dendritic cells: the effectiveness of lymphokine-activated killer (LAK) cells, as well as of peripheral blood and monocyte-derived dendritic cells for immunotherapy of cancer was investigated in vitro, and were tested in a clinical trial for the treatment of patients with gastrointestinal cancer and brain tumors.
4) Development of new vaccines targeting the tumor angiogenesis for the treatment of cancer: new vaccine strategies for the treatment of solid tumors by targeting the tumor vasculature are being developed at the department. The vaccine, tested in an animal model of colon cancer metastasis, showed to be very effective, and is being prepared for the clinical trial.
5) Vaccination therapy of patients with habitual abortion using the husband's lymphocytes, and investigation on their mechanism of action.
6) Effects of radiation and filtration on platelet function: the effects of radiation and the use of white blood cell (WBC)-reduction filters on the platelet function (adhesion and aggregation) have been studied. Irradiation did not affect the platelet function, but the WBC-reduction filters, depending on the type of filter, reduced the adhesive capacity of platelets.
7) Development of a new methodology for the evaluation of platelet function: a new method, based on the adhesive capacity of platelets, is being developed at the department.
8) Peripheral blood stem cells transplantation: peripheral blood stem cells are isolated at the department for transplantation in leukemic patients.

Current Research

The research projects described above are still ongoing at the department of Transfusion Medicine, which are:

1) Human platelet antigen/antibody detection and characterization, and study on their role in transfusion practice;
2) Human endothelial cell antigen/antibody detection and characterization, and study on their role in the pathogenesis of inflammatory and autoimmune diseases, as well as in organ transplantation;
3) Immunotherapy of cancer using LAK cells and dendritic cells;

4) Development of new vaccines targeting the tumor angiogenesis for the treatment of cancer;
5) Lymphocyte vaccination therapy for patients with habitual abortion;
6) Effects of radiation and filtration on platelet function;
7) Development of a new methodology for the evaluation of platelet function;
Peripheral blood stem cells transplantation.

Future Prospects

The future prospects of the department are as follows:
1) To develop the ideal method of platelet collection and preservation for transfusion, especially related to preservation of platelets without loss of their function;
2) To develop a good methodology for the evaluation of platelet function;
3) To develop a new therapeutic strategy to combat cancer by targeting the angiogenic vasculature of tumors;
4) To develop the vascular regeneration medicine using peripheral blood and cord blood stem cells.

Research Grants

(1) Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan –Kiban (C)(2); Development of a new methodology for the detection of anti-endothelial cell antibodies and evaluation on their clinical significance. 1997 – 1998;3 million yen.
(2) Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (B)(2): Development of a monoclonal antibody-based strategy for cancer treatment targeting the angiogenic vascular endothelium. 1999 – 2001;11,800 thousand yen.
(3) Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan- Grant for Highly-advanced Medicine, Development of a cell-based and gene-based strategies for the treatment of malignant and other life-threatening disorder of difficult control. 1998 – 2000; 131,304 thousand yen.

Select Publications

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10. Sunami E, Tsuno NH, Kitayama J, Osada T, Saito S, Tomozawa S, Tsuruo T, Shibata Y, Nagawa H. Decreased Synthesis of MMP-7 and Adhesion to Extracellular Matrix Proteins of Human Colon Cancer Cells Treated with Troglitazone. Surgery Today 2001
11. Tomozawa S, Tsuno NH, Sunami E, Hatano K, Kitayama J, Osada T, Saito S, Tsuruo T, Shibata Y, Nagawa H. Cyclooxygenase-2 overexpression correlates with tumour recurrence, especially haematogenous metastasis, of colorectal cancer. Br J Cancer; 83(3):324-328, 2000.
12. Sunami E, Tsuno N, Osada T, Saito S, Kitayama J, Tomozawa S, Tsuruo T, Shibata Y, Muto T, Nagawa H. MMP-1 is a prognostic marker for hematogenous metastasis of colorectal cancer. Oncologist;5(2):108-14, 2000.
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patients with colorectal carcinoma. Cancer Jan 1;88(1):42-9, 2000.
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