Research Hospital

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We have been challenging to cure intractable hematological disorders such as leukemia and lymphoma mainly with the aid of hematopoietic stem cell transplantation (HSCT). No less than 40 patients per year receive allogeneic HSCT in our facilities. Based on our achievement as a main hub of HSCT centers in Japan, we greatly contributed to establish the Japan Marrow Donor Program (JMDP) and have been continuously working for JMDP in not only transplantation but also collection of unrelated donor marrows. In recent years, unrelated cord blood has turned to be our major stem cell source in HSCT. Since 1998 we have performed up to 50 cases of unrelated CBT for adult patients, which appears a distinguished experience in the world.

1. Unrelated cord blood transplantation for adult patients with advanced myelodysplastic syndrome

J Ooi, T Iseki, S Takahashi, et al.

We report the results of unrelated cord blood transplantation (CBT) for 13 adult patients with advanced MDS. The median age was 40 years, the median weight was 51 kg, and the median number of infused nucleated cells was 2.43 x 10^7/kg. Twelve patients had myeloid reconstitution and the median time to >5 x 10^8/l absolute neutrophil count was 22.5 days. A self-sustained platelet count greater than 50 x 10^9/l was achieved in 11 patients at a median time of 49 days. Acute GVHD occurred in 9 out of 12 evaluable patients and chronic GVHD in 8 out of 11 evaluable patients. Ten patients are alive and free of disease at between 171 and 1558 days after transplantation. The probability of disease-free survival at 2 years was 76.2%. These results suggest that adult advanced MDS patients without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.

2. Cytomegalovirus infection following unrelated cord blood transplantation for adult patients in IMSUT research hospital

A Tomonari, T Iseki, J Ooi, S Takahashi, et al.

Cytomegalovirus (CMV) infection in 28 adult patients after cord blood transplantation (CBT) from unrelated donors was compared with that after bone marrow transplantation from HLA-matched related (R-BMT) and unrelated (U-BMT) donors. Positive

CMV antigenemia was seen in 19 (79%) of 24 CMV-seropositive patients at a median of 42 days (range, 29 to 85) after CBT, but in 0 of 4 CMV-seronegative patients. This probability did not differ significantly from those after R-BMT and U-BMT (66%, $P = 0.22$ and 60%, $P = 0.15$, respectively). Based on the antigenemia results, 16 patients (67%) received pre-emptive ganciclovir therapy from a median of 47 days (range, 36 to 67) after CBT. This probability was higher than that after R-BMT (28%, $P = 0.0048$), but did not differ from that after U-BMT (50%, $P = 0.21$). In addition, the probability of requiring more than 2 courses of ganciclovir therapy after CBT (21%) was higher than those after R-BMT and U-BMT (0%, $P = 0.015$ and 0.039, respectively). One patient (5%) developed CMV disease after U-BMT, while no patients developed CMV disease after CBT or R-BMT. CMV serostatus, use of steroid, and HLA disparity affected the probability of requiring ganciclovir therapy after CBT ($P = 0.024$, 0.032, and 0.017, respectively). These results suggest that recovery of CMV-specific immunity after CBT is delayed when compared with BMT.

3. Varicella-zoster virus infection in adult patients after unrelated cord blood transplantation in IMSUT research hospital

A Tomonari, T Iseki, S Takahashi, J Ooi, et al.

Varicella-zoster virus (VZV) infection in 33 adult patients who underwent cord blood transplantation (CBT) from unrelated donors between 1998 and 2002 was studied. Nineteen (58%) of 33 patients developed VZV reactivation at a median of 3.7 months after CBT (range, 1.7 to 24). Eighteen patients developed localized herpes zoster and 1 had varicella with visceral dissemination. All the patients responded well to antiviral therapy. The cumulative incidence of VZV reactivation after CBT did not differ significantly from that after bone marrow transplantation (BMT) from HLA-matched sibling donors (69% *vs* 45%, $P = 0.15$). However, the probabilities of VZV reactivation after CBT by 90 and 120 days were significantly higher than after BMT (21% *vs* 0%, $P = 0.0097$ and 30% *vs* 7%, $P = 0.023$, respectively). The absence of grade II-IV acute graft-*versus*-host disease (91% *vs* 25%, $P = 0.003$) and prior chemotherapy for lymphoid malignancies (89% *vs* 59%, $P = 0.0063$) were associated with higher rates of VZV reactivation after CBT. These results suggest that prolonged antiviral prophylaxis to reduce the early VZV reactivation is advantageous for high-risk patients after CBT.

4. Effect of cyclophosphamide on serum cyclosporine levels at the conditioning of hematopoietic stem cell transplantation

F Nagamura, T Takahashi, et al.

We retrospectively analyzed the factors which may affect serum CsA concentrations from day 0 to day14 of allogeneic hematopoietic stem cell transplantation (HSCT). One hundred three recipients who received short term MTX and CsA for prophylaxis of acute GVHD were analyzed. No significant relationships between serum CsA concentrations and gender, age, serum creatinine level, AST/ALT levels, and antibiotic administration, and fluconazole administration were observed. Median serum CsA concentration of CY-containing regimen was significantly lower than that of non-CY regimen. The mean of median CsA concentrations of CY-containing regimen (n=54) was 146.0 ng/ml (95% C.I.: 131.8-160.2), while that of non-CY regimen (n=49) was 211.2 ng/ml (189.3-233.1) ($p<0.0001$). By a longitudinal analysis, the difference between the two regimen groups at day 0 was significant, and significant differences continued until day 14. This result was also supported by the analysis for each conditioning regimen. Means of median serum CsA concentrations in TBI + CY regimen and TBI + G-CSF combined Ara-C (G/Ara) + CY were significantly lower than those of TBI + G/Ara and TBI + etoposide. Our results suggest that CY at conditioning has the effect of reducing serum CsA concentrations at least during the early period after HSCT.

5. Sustained B cell depletion results from maturation arrest at a proB cell stage in a SCT recipient with extensive chronic GvHD; implication of Pax5 deficiency

A Tojo, R Sekine, et al.

B cell reconstitution after allogeneic stem cell transplantation (SCT) is delayed in patients with chronic graft versus host disease (cGvHD). The quantitative B cell deficiency is partly attributable to decreased B cell production (B lymphopoiesis) in the marrow of SCT recipients with cGvHD (Storek J et al., 1998). However, little is known about at which stage B lymphopoiesis is inhibited by GvHD and/or its treatment. We extensively analyzed B cell precursors in the marrow of a SCT recipient who has been suffered from extensive cGvHD and severely impaired in immunoglobulin production for 8 years after transplantation. We found that his B cell development is arrested at a proB cell stage, characterized by lack of Pax5 transcripts. A 24 y/o male patient (Pt) with chronic myeloid leukemia (CML) underwent bone marrow transplantation from his HLA-

matched healthy sibling donor in 1994. He was conditioned with a total body irradiation-containing regimen and received cyclosporine A (CSA) and short-term methotrexate for GvHD prophylaxis. He developed grade IV acute GvHD, followed by extensive cGvHD which was refractory to CSA and prednisone (PSL). He has systemic skin lesions including rash, erosion, pigmentation and depigmentation but is free from liver and gut cGvHD. The pathological finding of the biopsy specimen indicated massive skin infiltration of T cells. Because of renal toxicity, CSA was discontinued in 1997 and PSL was gradually tapered. Instead, systemic application of FK506 ointment and oral mycophenolate mophetil was started in 2001. He has been requiring biweekly or monthly replacement of immunoglobulin to maintain serum IgG level at 3.0 g/L until now. He revealed the negligible level of circulating and marrow $CD19^+$ cells (< 0.02% of lymphocytes), whereas the absolute number of circulating $CD3^+$, $CD4^+$ or $CD8^+$ cells was comparable to that of healthy individuals, respectively. We determined the relative amount of B cell precursors in the marrow of the patient and an age-matched SCT recipient without cGvHD (control) by multi-color flowcytometry. The results were indicated as percentage of T cell-depleted lymphocytes. $CD19^+VpreB^+$ cells: 0.01 (Pt) *v.s.* 4.8 (Ctrl), IgM^+VpreB^+ cells: 0.02 *v.s.* 4.1, TdT^+VpreB^+ cells: 1.4 *v.s.* 2.4, IgM^+CD79a^+ cells: 0.00 *v.s.* 33. This finding suggests a paucity of more mature B cell precursors than CD19-TdT+$VpreB^+$ (early proB) cells in the marrow. B cell development around this stage is primarily controlled by a B cell specific activator protein, Pax5. RT-PCR analysis performed on his bone marrow mononuclear cells demonstrated that E2A and RAG-1 but not Pax5 transcripts could be detected. Sustained B cell deficiency results from maturation arrest within a proB cell compartment, characterized by lack of Pax5 transcripts, in a CML Patient with extensive chronic GvHD after allogeneic BMT. It is still unclear whether Pax5 deficiency is associated with cGvHD and how Pax5 expression is suppressed.

6. Efficacy and safety of fludarabine and dexamethasone combined with rituximab in the treatment of relapsed and/or refractory non-Hodgkin's lymphoma

K Uchimaru, A Tojo, et al.

Treatment strategy for relapsed low-grade non-Hodgkin's lymphoma (NHL) is still controversial although a number of promising therapeutic options including allogeneic stem cell transplantation and anti-CD20 monoclonal antibody (rituximab) have been available up to now. Fludarabine, a purine analog, exerts a potent activity against lymphoid malignancies exhibiting indolent growth profiles, and there is some evidence for synergism between fludarabine and rituximab in anti-tumor effects on CD20-positive B cell lymphomas. In the present study, we performed a combination therapy consisting of fludarabine, rituximab and dexamethasone (FRD) in patients with B cell lymphoma which were all positive for CD20 and relapsed after CHOP regimen. 7 patients (male/female ratio = 4/3, average 53.0 y/o) were enrolled in the pilot study. Pathological findings were 5 follicular center, 1 diffuse large, and 1 diffuse mixed type. Disease activities were 2 1st relapse, 2 2nd relapse, 1 3rd relapse, and 2 primary refractory cases. 5 patients proved to be at stage IV, and the other two cases were at stage II and III, respectively. Treatment protocol comprises 6 cycles of fludarabine 25mg/m^2 (day 1-3), dexamethasone 20 mg/m^2 (day 1-3) and rituximab 375mg/m^2 (day 3) with 3 weeks' interval. As a result, 5 patients with follicular lymphoma achieved complete remission although one case was also treated with local irradiation. One diffuse lymphoma patients revealed partial remission. Adverse events including severe neutropenia (1), palmar and plantar erythema (2), and peripheral neuropathy were observed. When given together with prophylaxis for Pneumocystis carinii, infectious complications with FRD have been quite modest. In conclusion, FRD would be a well-tolerated and effective regimen for the treatment of relapsed and/or refractory low-grade NHL.

7. One allele deletion of the RB1 gene in a case of MDS RA with del (13)(q12q14): fluorescence In situ hybridization study of the RB1 gene

F Nagamura, K Uchimaru, et al.

The tumor suppressor gene, RB1, is known to be located on chromosome band13q14. We investigated the involvement of the RB1 gene in a case of myelodysplastic syndrome - refractory anemia with del(13)(q12q14) by florescence in situ hybridization (FISH) analysis using the RB1 locus (13q14) DNA probe. Bone marrow cells derived from this patient exhibited a single signal of the RB1 gene in 58/100 bone marrow cells as determined by interface FISH analysis. Hematopoietic colony forming assays showed that the absolute number of erythroid, myeloid and mixed colonies were comparable to those observed in normal subjects. FISH analysis of selected colonies revealed that only a single signal for the RB1 gene was detected in 5 out of 5 CFU-GM, 4 out of 5 BFU-E and 2 out of 4 CFU-Mix (total 11/14: 78.6%). Thus, the majority of hematopoietic progenitor cells lacked one allele of the RB1 gene, suggesting that in this particular case, the RB1 gene played an important role in abnormal hematopoiesis.

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Research Hospital

Department of Infectious Diseases and Applied Immunology

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Department of Infectious Diseases and Applied Immunology (DIDAI) was founded in 1981. In 1986, clinic for patients with human immunodeficiency virus (HIV) infection was opened by former professor, K. Shimada. In 2002, approximately 180 patients with HIV infection visit the out-patient clinic on a monthly basis, and 3-5 beds for HIV-infected patients in the in-patient ward are usually occupied. Since the number of the staff members of DIDAI is too small to care both out-patients and in-patients, members of the Division of Infectious Diseases (DID) and the Division of Clinical Immunology of the Advanced Clinical Research Center join the clinic. Supported by clinicians of three department and divisions, basic scientists of immunology and virology in DID, and dedicated medical and paramedcal stuffs, IMSUT hospital provides the most up-to-date medical treatment to HIV-infected patients in Japan. DIDAI is also a treatment center for international infectious diseases such as malaria and typhoid fever.

1. Treatment of and clinical research on HIV-infection and related diseases

Tetsuya Nakamura, Takashi Odawara[1], Takashi Takahashi[1], Hitomi Nakamura[1], Tomohiko Koibuchi[1], Toshiyuki Miura[1], Tokiomi Endoh[1], Miou Sato[1], Akihiro Hitani[1], Mieko Goto[1] and Aikichi Iwamoto[1]: [1]Department of Infectious Diseases

a. Treatment of HIV infection in IMSUT hospital

i. Statistical characteristics of HIV-infected patients in IMSUT hospital this year

Twenty-nine new patients with HIV-1 infection visited our hospital this year, and as of the end of this year, 179 patients in total are under medical management in our out-patient clinic. As shown in the figure below, the number of total patients declined in 1997 because a part of patients as well as medical stuffs were moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again with somewhat exponential curve afetr 1998 in accordance with Japanese statistics of HIV-infected patients. In contrast, the number of admission has decreased since 1997 because of the introduction of highly active anti-retroviral therapy (HAART) which effectively suppresses the replication of HIV. After one year of HAART, the viral loads become unde-

tectable in more than 90% of patients, and their CD4 counts increase by approximately 200/microL in average. Consequently, the clinical management of HIV-infected patients changed from how to treat opportunistic infections into how to keep patients under HAART.

ii. A Randomized, Open-Label, Phase III, International Study of Subcutaneous Recombinant IL-2 (Proleukin) in Patients With HIV-1 Infection and CD4+ Cell Counts ≥ 300/mm3 : Evaluation of Subcutaneous Proleukin in a Randomized International Trial (ESPRIT)

We joined last year the international clinical study called "ESPRIT" which is organized by National Cancer Institute in US. Approximately 4, 000 subjects are scheduled to participate in this study from around the world. The study is scheduled to be conducted over the course of 6 years and ten patients are assigned to IMSUT hospital. We continued the clinical study this year, too and one patient was enrolled in this study.

IL-2 is a substance that is normally produced in the body, and act to increase CD4 cells. CD4 cells assist in defense against infection. The concentrations of IL-2 produced in patients with HIV infection have been determined to be lower than in normal persons. The object of this study is to determine whether bringing about an increase in the number of CD4 cells by administration of IL-2 leads to a decrease in the incidence of onset of HIV-related diseases. In clinical studies conducted thus far, increases in CD4 cells have been observed in the vast majority (but not all) patients administered IL-2. It is still unknown, however, whether these increases really improved the health of the patients. This study will be conducted in order to investigate the following matters.

- Whether or not IL-2 reduces serious infections related to HIV and prolongs the survival period in the case it is used concomitantly with other HIV drugs.
- Whether or not IL-2 can be administered safely over an extended period of time to HIV infection patients.

b. Clinical research on Infectious Diseases

i. A case report of disseminated coccidioidomycosis

We report a Japanese case of paravertebral and intravertebral abscesses caused by *Coccidioides immitis*. The patient had stayed in Arizona, USA, for five years, and was suffered from overt disease after coming back to Japan. Culture of the pus from paravertebral abscess revealed *Coccidioides immitis*, and the diagnosis of disseminated coccidioidomycosis was made. Oral fluconazole (600 mg/day) was started, and the abscesses surrounding vertebral bodies disappeared after two years of treatment. The abscess in the vertebral bodies also responded to treatment, but the small lesion was still left in the 10th vertebral body after two years of treatment. Coccidioidomycosis is a fungal infection that is endemic in southwestern United States and central and south America. Although coccidioidomycosis causes self-limiting flulike illness or pneumonia, a small proportion of the infection progresses to disseminated diseases. Since the incidence of coccidioidomycosis is increasing year by year, physicians in not only endemic but nonendemic areas have to think of coccidioidomycosis as one of the differential diagnoses when they examine patients from endemic areas.

ii Dihydrofolate reductase gene polymorphisms in Pneumocystis carinii

We examined polymorphisms in dihydrofolate reductase (DHFR) gene of *Pneumocystis carinii* isolated from 27 patients with *P. carinii* pneumonia (PCP) in Japan. We found 4 substitution sites with 2 synonymous and 2 non-synonymous changes. Two synonymous substitutions at nucleotide positions 540 and 312 were identified in one and thirteen patients, respectively. Two amino acid substitutions (Ala67Val, Cys166Tyr) were found in two different patients. No linkage of amino acid substitutions in DHFR to those in dihydropteroate synthase was observed. The two patients whose isolates showed non-synonymous DHFR mutations were not exposed to DHFR inhibitors before they developed PCP and were successfully treated with co-trimoxazole.

2. Diagnosis and Treatment of Tropical Diseases

Tetsuya Nakamura, Takashi Odawara[1], Takashi Takahashi[1], Hitomi Nakamura[1], Tomohiko Koibuchi[1], Toshiyuki Miura[1], Tokiomi Endoh[1], Miou Sato[1], Akihiro Hitani[1], Mieko Goto[1] and Aikichi Iwamoto[1]: [1]Department of Infectious Diseases

This year, we treated patients with malaria, dengue fever, tape worm, liver abscess, typhoid fever and trichinosis. Since Ministry of Health, Labour and Welfare approved mefloquine for prophylaxis of malaria in September 2001, the number of people who plan to travel to areas endemic of malaria and visit IMSUT Hospital for mefloquine prescription is increasing. We not only have treated patients with tropical diseases but also have accepted consultations via telephone and E-mails from people who travel in tropical areas. We will continue this consultation activity in addition to mefloquine prescription as prophylaxis of malaria in out-patient clinic.

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Our major goal is to cure children suffering from a variety of life-threatening hematological disorders. Attempting to achieve this, we continue the commitment to treatment and follow-up care of such children, and to clinical and laboratory research that ultimately will help us devise better therapeutic approaches to these deseases. Currently efforts are directed toward hematopoietic stem cell transplantation including ex vivo expansion of human hematopoietic stem cells, gene therapy, immunotherapy and analysis of pathogenesis of hematopoietic disorders.

1. Hematopoietic stem cell transplantation for children with high-risk leukemia

Yasuhiro Ebihara, Daisuke Hasegawa, Yoshitoshi Ohtsuka, Toshihisa Tsuruta, Atsushi Manabe, Kohichiro Tsuji

Although a standard regimen in hematopoietic stem cell transplantation (SCT) has been available for children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), it has not been standardized for those with rare diseases including congenital bone marrow failure syndrome (CBMFS) and natural killer cell leukemia. A multi-institutional trial using regimens with a rationale should be proposed in a prospective manner. For CBMFS, we conducted in vitro and in vivo assays to assess the sensitivity of leukemic cells to G-CSF and transplanted 3 patients whose leukemic cells had a high sensitivity to G-CSF using a regime including G-CSF. Thus, we could avoid intensive chemotherapy before SCT for patients with a vulnerable normal bone marrow reserve. For patients with Fanconi anemia, we employed a regimen containing fludarabine to reduce the dose of alkylating agents and irradiation to avoid the toxicity, which was otherwise likely to occur in those patients. For patients with NK cell diseases, we used a regimen combining alkylating agents (cyclophosphamide and thiotepa) and total body irradiation based on the results that NK leukemic cells strongly expressed multidrug-resistant genes in 2 patients. Now we plan to extend our experience in nationwide collaborative studies.

2. Pediatric myelodysplastic syndrome

Atsushi Manabe, Yasuhiro Ebihara, Daisuke Hasegawa, Yoshitoshi Ohtsuka, Toshihisa Tsuruta, Kohichiro Tsuji

Pediatric myelodysplastic syndrome (MDS) is a rare disease and only 50-100 children under the age of 16 suffer from the disease annually. The diagnosis and treatment has not been standardized and it should be determined in a nationwide manner. The MDS committee of the Japanese Society of Pediatric Hematology began the pathologic central review in 1999 and we reviewed all the samples of patients sus-

pected of having MDS. At present, over 150 patients have been enrolled and standard diagnostic criteria have been proposed for juvenile myelomonocytic leukemia (JMML), a subset of MDS. We also tested in vitro cell growth for patients with JMML using diagnostic samples. The results showed that spontaneous growth and hypersensitivity to GM-CSF were observed in most children with JMML. We proposed a cooperative trial to establish the treatment for MDS (MDS99) and have enrolled over 50 patients from the whole country.

3. Cancer immunotherapy in children

Atsushi Manabe, Imiko Hirose, Kohichiro Tsuji, Naohide Yamashita

Although a notable improvement of cure rate in childhood cancer has been achieved by the development of multiagent chemotherapy, approximately one half of children with cancer do not survive with a contemporary treatment. Even a mega-dose chemotherapy combined with stem cell support cannot eradicate the disease completely in this subpopulation of patients. A novel approach is needed for these patients. Recently, cancer immunotherapy employing a gene therapy technique was proposed. Actually, gene therapy using autologous tumor cells transduced with GM-CSF has been performed for renal cell cancer patients in this institute. The aim of this project is to establish a feasibility of this modality in treating retractable cancer in children.

Neuroblastoma (NB) is one of the most frequent solid tumors in children and NB in children over one-year-old is known to be very difficult to cure even with stem cell transplantation. It has been observed that NB in some infants regresses spontaneously and its mechanism has not yet been elucidated. NB is derived from a neural crest which also derives malignant melanoma for which immunotherapy has already been established after identification of melanoma-associated tumor specific antigens such as gp100, MART-1/Melan-A, tyrosinase and MAGE-1. It is possible that some immunological mechanism may play a role in the behavior of infant NB.

Currently, we are preparing a gene therapy for recurrent neuroblastoma. In collaboration with Dr. Malcolm Brenner at Baylor College in Houston, USA, we plan to use a tumor vaccine transduced with IL-2 and lymphotactin. It was shown that the vaccination of neuroblastoma cells transduced with IL-2 into patients with relapsed neuroblastoma exerted regression of tumors. It is possible that gene-transduced neuroblastoma cells may become immunogenic and evoke an antitumor effect of the body. The addition of lymphotactin to IL-2 is expected to attract lymphocytes to the vaccinated neuroblastoma cells. Preclinical experiments showed the feasibility of this therapy. The institutional review board and the committees of the government already approved the protocol. We started enrollment of the patients.

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Our section was founded in 2001 *for management of patients with various autoimmune/allergic diseases. The goals of our team are to:*
Improve the standards of treatment of allergy and clinical immunology
Encourage research in the field of allergy and clinical immunology. We participate in cutting edge studies of the newest treatments for autoimmune, rheumatic and allergic diseases. In addition to conventional drug studies aimed to improve the efficacy and safety of current therapies, we are going to carry out experimental protocols of particular interest for patients not responding to conventional therapy. For that purpose we have been collaborating with the Division of Clinical Immunology (Prof. Chikao Morimoto).

1. Therapeutically targeting transcription factors

Hirotoshi Tanaka, Yuichi Makino, Noritada Yoshikawa, Noriaki Shimizu, Tsunenori Kodama, Rika Ouchida, Hiroshi Nakamura, Tetsuya Hisada, Chikao Morimoto (Division of Clinical Immunology), Hiroshi Handa (TokyoInstitute of Technology), Masatoshi Kusuhara, Fumitaka Ohsuzu (National Defence Medical College) (in collaboration with Lorenz Poellinger Lab, Karolinska Institute, Sweden)

We are interested in the mechanism of eukaryotic gene expression and development of novel therapy and/or drug which target transcriptional machineries. For this purpose, our recent work is mainly focused on conditional regulation of transcription factors including the glucocorticoid receptor and hypoxia-inducible factor-1α.

a. Glucocorticoid receptor project

Glucocorticoid hormones are effective in controlling inflammation, but the mechanisms that confer this action are largely unknown. It has been shown that both positive and negative regulation of gene expression are necessary for this process. The genes whose activity is negatively modulated by glucocorticoids in the inflammatory process code for several cytokines, adhesion molecules. Most of them do not carry a classical binding site for regulation by the glucocorticoid receptor (GR), but have instead regulatory sequences for transcription factors such as AP-1 or NF-κB. Considering various severe side effects of glucocorticoids, it may be pharmacologically important to dissociate these negative regulatory function of the GR from induction of metabolic enzymes, gene expression of which has been shown to be positively regulated by the GR. We propose that a certain class of compounds (surprisingly, some of them are nonsteridal chemicals) may dissociate transactivation and transrepression function of the GR and offer opportunities for the design of such compounds that could function more effectively as antiinflammatory drugs. In this line, we are developing the strategy for identification of novel therapeutic bullets.

(i) Redox Regulation of the Glucocorticoid Receptor

Redox regulation is currently considered as a mode of signal transduction for coordinated regulation of a variety of cellular processes. Transcriptional regulation of gene expression is also influenced by cellular redox state, most possibly through the oxido-reductive modification of transcription factors. The GR belongs to a nuclear receptor superfamily and acts as a ligand-dependent transcription factor. We demonstrated that the GR function is regulated via redox-dependent mechanisms at multiple levels. Moreover, it is suggested that redox regulation of the receptor function is one of dynamic cellular responses to environmental stimuli and plays an important role in orchestrated crosstalk between central and peripheral stress responses.

(ii) Development of Dissociating Ligand for the Glucocorticoid Receptor

The GR function could be differencially regulated by ligands. We have recently shown that not only synthetic glucocorticoids but also certain bile acids could differentially modulate GR function. Moreover, the effects of those compounds are indicated to be ascrived to the ligand binding domain of the receptor. In this line, we are going to isolate the dissociating ligand that preferencially promotes transrepression function of the GR.

On the other hand, receptor specificity is another important aspect of novel GR regulator. In this line, we have shown that cortivazol is extremely specific for GR and does not bind to MR. We are studying the molecular basis for this receptor specificity of the ligand using cortivazol as a model.

(iii) Molecular biology of a novel protein HEXIM1

We have recently cloned the cDNA encoding a novel protein HEXIM1, expression of which is induced by treatment of vascular smooth muscle cells with a differentiation inducer hexamethylane bisacetamide. We showed that HEXIM1 is a nuclear protein and represses NF-κB-dependent transcription. Since NF-κB plays a pathological role in smooth muscle cell proliferation, our study will not only unveil pathogenesis of but also contribute to therapy of atherosclerotic vascular disorders.

(iv)Hypoxia-inducible Factor (HIF)-1a project

HIF-1α is essential for not only angiogenesis but also development of certain organs. In this line, molecular biology of HIF-1α will provide us possible advantage to characterize and manupilate such processes.

b. Transcriptional Network Controlling Angiogenesis in Health and Diseases

Angiogenesis is regulated by a combination of various factors including transcription factors. Recently, we have isolated cDNA encoding the novel protein IPAS which can squelch HIF-1α. Its tissue-specific expression argues the physiological role of transcriptional network for orchestrated regulation of angiogenesis. We are currently studying the molecular mechanism of the interaction between HIF-1α and IPAS. This negative regulator may also therapeutically applicable for treating a number of angiogenic disorders including cancer, diabetic retinopathy, and rheumatoid arthritis. Moreover, we have recently shown that IPAS is a splice variant of HIF-3α, and its mRNA expression is enhanced under hypoxic conditions. This conditional regulation of splicing is our current interest.

On the other hand, we have recently identified that HIF-1α function is regulated in a various fashion in certain physiological settings, which may be important for homeostatic control of tissue function. In this line, we are now identifying the molecular mechanism for such regulation of HIF-1α.

2. Study on serum soluble CD26 in patients with systemic lupus erythematosus

Osamu Hosono, Hiroshi Kobayashi, Hiroshi Kawasaki, Hirotoshi Tanaka, Chikao Morimoto

CD26 is the cell surface activation antigen with dipeptidyl peptidase IV (DPPIV) enzyme activity that is preferentially expressed on memory T cells and has a role in T cell immune responses. The soluble form of CD26 is present in serum and recombinant soluble CD26 can enhance in vitro antigen-specific T cell responses. To determine the role of soluble CD26 (sCD26) in the pathophysiology of patients with systemic lupus erythematosus (SLE), we measured levels of sCD26 and its specific DPPIV activity in serum. Serum levels of sCD26 and its specific DPPIV activity were significantly decreased in SLE and were inversely correlated with SLE disease activity index score, but not with clinical variables or clinical subsets of SLE. Close correlation between sCD26/DPPIV and disease activity was observed in the longitudinal study. Serum levels of sCD26 may be involved in the pathophysiology of SLE, and appear to be useful as a new disease activity measure for SLE.

3. Study on chemokine receptor function in synovial fluid T cells from patients with rheumatoid arthritis

Osamu Hosono, Naoko Hisakawa, Hirotoshi Tanaka, Chikao Morimoto

To study expression and function of the chemokine receptor CCR5 in synovial fluid (SF) T cells from patients with rheumatoid arthritis (RA). SF T cells

showed an increase in the population of CCR5, CXCR4, and CD45RO positive cells and exhibited an increase in chemotactic activity, which was not augmented with RANTES but stromal cell-derived factor-1alpha. Tyrosine phosphorylation per CasL molecule was markedly enhanced in SF T cells. In H9 cells, tyrosine phosphorylation of not only focal adhesion kinase but also CasL was induced after treatment with RANTES. Downmodulation of CCR5 by RANTES was decreased and recycling of CCR5 was accelerated in SF T cells when compared with peripheral blood (PB) T cells. When CD45RO positive PB T cells were cultured with interleukin 2, blunted responsiveness to RANTES-induced chemotaxis was reproduced as well as spontaneous chemotaxis, increased expression of CCR5, and aberrant receptor dynamics, after RANTES stimulation as observed in SF T cells. Synovial fluid T cells highly positive for CCR5 show aberrant characteristics; resistant to RANTES in terms of migration, but responsive in terms of dynamics of CCR5.

4. Basic and clinical immunology of chemokine / chemokine receptors

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We have been pursuing the structure and functional analysis of human chemokine/chemokine receptor system in order to clearly identify their roles in innate and acquired immune system. Since the discovery of chemokine receptors as the HIV co-receptors, this area of immune mediators have drawn tremendous attention. We previously showed that CCR-1 and CCR-3 are two of the main regulators in the induction of allo-immune response. Furthermore, we have shown that the responsiveness of CCRs is intricately controlled by a panel of small GPTases. Of note was that chemokines were powerful mediators of activation of dendritic cells in the process of antigen presentation. These observations were extended to address several clinical issues such as skin inflammation and ocular autoimmune disease. Consequently we believe manipulation of chemokine system would be a tool to activate or regulate immune responses.

Monoclonal antibodies could be an attractive tool to that end. Their targets are clearly characterized and strict pharmacological monitoring is possible. Several attempts were conducted to eliminate inherent rodent-origin antigenicity, including chimerization with human Ig and CDR grafting. In fact, antibodies to CD20 and *Neu* oncogene have been in clinical use with successful results. A key feature of humanizing strategies has been to leave many of the framework residues as found in the rodent rather than the human counterpart. The drawback of this approach is that the resultant antibodies may ultimately elicit an undesirable anti-globulin response when administered to patients, and may induce the production of neutralizing anti-antibodies, resulting in the disappearance of original specificity and efficacy.

Recently fully humanized rodent-origin antibodies became available by phage-display technology and trans-chromosomal (TC) mouse that possesses human chromosome encompassing immunoglobulin genes. TC mice drawn our attention since they were shown to produce agonistic, as well as antagonistic, antibodies in high frequency and were able to produce mouse-cross reactive antibodies by suppressing BCR-signaling pathways which enables us the further in vitro analysis. We are underway to develop TCmice-derived monoclonal antibodies to CCR-1 and CCR-3 since we believe CCR-1 and CCR-3 are theterapeutic targets for autoimmune diseases, systemic and organ-specific, transplant rejection and GVHD. We also believe that this approach could be a tool to efficiently induce anti-tumor immunity by elaborating agonistic antibodies.

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Department of Advanced Medical Science was established in September 1997. We are investigating (1) Adeno-Associated Virus-Mediated Gene Expression in Human Placenta-Derived Mesenchymal Cells, (2) Identification of genes involved in outflow tract formation during embryonic heart development, (3) Potential pro-angiogenic cell therapy for ischemic disease using hPDMCs, (4) Pyk2/RAFTK signaling in hMVEC (human micro vascular endothelial cells), (4) Immunotherapy for malignancies. We are planning and progressing several projects described below to develop a new therapy for several diseases, including carcinomas and ischemic disorders.

1. Adeno-associated virus-mediated gene expression in human placenta-derived mesenchymal cells

Nakaoka T. et al.

Mesenchymal cells from various sources are pluripotent and are attractive sources for cell transplantation. In case of placenta, the villous region contains the mesenchymal cell population. Meanwhile, adeno-associated virus (AAV) has gained attention as a potentially useful vector for human gene therapy mainly by virtue of its safety. Although recombinant AAV (rAAV) transduces gene into muscle and neuron efficiently *in vivo*, there are still controversies over using it as a vector for gene transduction into stem cells. If rAAV transduces suitable gene efficiently into the pluripotent cell, many therapeutic applications for cell transplantation would be warranted. Therefore, we analyzed rAAV-mediated gene expression in human placenta-derived mesenchymal cells (hPDMCs). After transduction of AV-CAG-EGFP, a rAAV expressing enhanced green fluorescence protein (EGFP), hPDMCs showed much higher level of EGFP expression than human umbilical vein endothelial cells (hUVECs) or rat aortic smooth muscle cells (rASMCs). The number of EGFP-positive hPDMCs infected by AV-CAG-EGFP alone did not increase significantly by coinfection of adenovirus, which enhanced expression level of the rAAV vector. Moreover, flow cytometric analysis showed discrete positive fraction of EGFP-expressing hPDMCs, which is about 15-20% of the cells infected with AV-CAG-EGFP. Therefore, some cell population in hPDMCs might be highly susceptible to rAAV-mediated gene transduction. In addition, stable EGFP expressions were observed in about 1% of hPDMCs infected with AV-CAG-EGFP at 4 weeks post-infection. If transgene expression lasts in pluripotent cells for long, expansion of the cells with transgene expression would be feasible. In case transplantation of hPDMCs would be of therapeutic value in treatment of certain disease, a feature such as easy rAAV-mediated gene expression may further make hPDMC therapy promising.

2. Identification of genes involved in outflow tract formation during embryonic heart development

Nakaoka T. et al.

The heart defect (hdf) mouse is a recessive lethal mouse that arose from a lacZ reporter containing transgene insertional mutation. Homozygous embryos die in uteri by embryonic 11. 5 dpc. The outlet portion of the heart tube fail to form and consequently the portion comprising ventricle and atrium are highly dilated. Meanwhile, Cspg2 was identified as one candidate gene disrupted by the transgene insertion in the hdf mouse line. In this study, in order to further address molecular mechanisms responsible for abnormal heart development in hdf mice, we performed suppressive subtractive hybridization. As a result, mRNA expression of one clone was judged to be decreased in hdf homo to the level of one-tenth the mRNA expression in the littermates. Northern analysis showed that its mRNA is some 10 kb in length and that its expression in adult mouse tissue was detected relatively abundantly in uterus and less abundantly in heart, lung and spleen. Since the special expression pattern of this gene fragment in embryonic mouse seemed to mimic LacZ expression in heterozygous mouse, it is likely that decrease in its expression might account for some developmental abnormality in hdf mice. Moreover, its strong expression in branchial arches in embryonic tissues might suggest its involvement in the formation of outlet portion in heart development.

3. Potential pro-angiogenic cell therapy for ischemic disease using hPDMCs

Nishishita T. et al.

After the establishment of Cord Blood Banks, more than 2, 000 cord blood transplantations have been performed throughout the world. In the processing of cord blood, adjacent placenta has been so far thrown away. Recently, the Department of Cell Processing IMS started preparation and characterization of human placenta-derived mesenchymal cells (hPDMCs), which are obtained from placental villi. One of the characteristics of placenta is that its high vascularity. So, in our laboratory, we explored the possibility that these cells might produce angiogenic cytokines and could be used for pro-angiogenic cell therapy. We measured VEGF in hPDMCs conditioned media by ELISA and found that a large amount of VEGF, comparable to the amount produced by cancer cells, is produced by hPDMCs. We confirmed this VEGF is biologically active. These cells can be cultured without serum. These cells are easily transfected with AAV or Adenovirus vectors. So we can possibly express another gene if VEGF alone is not potent enough. HPDMCs might be opt for cell therapy also for the following reasons: 1. They can be processed in a large amount from placenta of normal deliveries without any intervention. 2. HLA can be typed and bank of cells could be made together with cord blood cells.

In vivo studies to test the efficacy of hPDMCs injection to improve ischemic status are on-going now. We made an animal model for arterial occlusive disease, inducing unilateral hindlimb ischemia by binding the left femoral arteries and veins of SCID mice. We injected hPDMCs in the ischemic muscles. Subcutaneous blood perfusion was analyzed by using a laser doppler perfusion image analyzer. Using hPDMCs. Preliminary studies show hPDMCs injected animals show better improvement of ischemia.

4. Pyk2/RAFTK signaling in hMVEC (human micro vascular endothelial cells)

Kuwabara K. et al.

Pyk2/RAFTK is a cytoplasmic tyrosine kinase and a member of focal adhesion kinase gene family. Pyk2 is known to play a role downstream of a signaling through a chemokine receptor such as CXCR4. In order to elucidate the role of pyk2 in endothelial function, we analyzed HMVEC infected with adenovirus expressing two type of dominant negative pyk2, AxY402F and AxK457A. HMVEC infected with AxY402F showed higher migration activity than HMVEC infected with control adenovirus, whereas infection of AxY402F inhibited growth of HMVEC. ERK1/2 activation was also detected in HMVEC infected with AxY402F. Increased migratory activity of HMVEC infected with AxY402F was considered to be at least partly through ERK1/2 activation, since U0126, ERK1/2 inhibitor, inhibited migration of HMVEC infected with AxK457A. These data suggest anti-migratory signaling mechanisms in HMVEC.

5. Immunotherapy for malignancies

Yamashita N. et al.

We are trying new therapies to patients with malignancy. For advanced thyroid cancer patients (thyroid cancer with distant metastasis) we are performing phase I study of immunotherapy using dendritic cells (DCs). The procedure to make mature DCs is as follows: periferal mononuclear cells are corrected using apheresis. Adherent cells to culture dishes are collected and GM-CSF and IL-4 are added to culture medium to make immature DCs. Tumor lysate is applied to immature DCs and further cultured in the presence of TNF-α. Thus obtained mature DCs are intracutaneously injected to the patients once a week for the first 4 times. After that,

additional 4 injections are done every two weeks. In conjunction with application of DCs rIL-2 is subcutaneoulsy injected. After therapy clinical evaluations are done. Till now 6 patients are enrolled.

Another immunotherapy is gene therapy for neuroblastoma patients. The aims of this study are as follows; (1) to determine the safety up to four subcutaneous (SC) injections of autologous neuroblastoma cells, which have been genetically modified by adenoviral vectors to secrete lymphotactin and interleukin-2, (2) to determine the safety of up to eight (total) injections in patients who have received the first four injections without unacceptable toxicity and have evidence of stable disease or better after receiving these injections, (3) to determine whether MHC restricted or unrestricted antitumor immune responses are induced by SC injection of modified autologous neuroblastomas and the cell doses required to produce these effects, (4) to obtain preliminary data on the antitumor effects of this treatment regimen. The protocol has been approved and we are enrolling the patients.

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We have been engaged in the surgical treatment of solid tumors and the immunotherapy of various malignancies. We have also been offering services, including upper and lower endoscopic examination, ultrasonic examination, and angiography. The principal goal of our department is to develop and conduct clinical trials in the early stages (Phase I and II) on patients at Research Hospital. We have performed phase I clinical trials of melanoma vaccine using gp100 derived peptides and have initiated cancer immunotherapy using dendritic cells.

1. Summary of surgical treatment and other procedures performed in 2002

Hideaki Tahara, Takuya Tsunoda, Yoshifumi Beck, Yasutaka Takeda, Iwao Yoshizaki, Takuya Takayama, Yuichi Ando, Naoya Ichikawa, Hiroaki Tanaka, Juichiro Konisi, Naomi Kimura, Norihiro Kokudo[1], Masatoshi Makuuchi[1]

[1] Division of Hepato-Biliary-Pancreatic Surgery and Artificial Organ and Transplantation, Department of Surgery, Graduate School of Medicine, University of Tokyo, Japan.

Surgical operations have been performed in 82 cases under general anesthesia and spinal or epidural anesthesia. As shown in Table 1, major operations were performed in 50 patients with malignant diseases and in 32 patients with benign diseases.

Procedures other than surgical operations performed in 2002 were as follows: angiography including trans-arterial embolization and trans-arterial chemotherapy (29 cases), gastroduodenal endoscopy (459 cases), and colorectal endoscopy (188 cases).

Table 1. Major operations performed in 2002

Malignant Diseases		Benign Diseases	
Cancer of the stomach	15	Cholelithiasis	10
Cancer of the colo-rectum	15	Inguinal hernia	9
Cancer of the bile duct	1	Miscellaneous	13
Cancer of the pancreas	4		
Cancer of the kidney	1		
Cancer of the breast	8		
Cancer of the esophagus	1		
Cancer of the thyroid	4		
Miscellaneous	1		
Total	50	Total	32

2. Phase I clinical trial of melanoma vaccine using gp100 derived peptides restricted to HLA-A*0201 or -A*2402

Takuya Tsunoda, Takuya Takayama, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Naomi Kimura, Hiroyuki Mushiake, Toshiyuki Baba, Shogo Nakano, Kenichi Ohyama, Hideaki Tahara

Epitope peptides derived from gp100, a melanoma associated antigen, are used for the cancer vaccine to treat the patients with advanced malignant melanoma. Patients with HLA-A*0201 were treated with a gp100 derived peptide (ITDQVPFSV) and another peptide with a mutation (IMDQVPFSV). Patients with HLA-A*2402, were treated with a gp100 derived peptide (VYFFLPDHL). All of the peptides were used with IFA in order to augment for anti-tumor immunity. To analyze the immune response of the vaccinated patients, HLA-Tetramer was prepared and used for staining of the peripheral blood lymphocytes taken from the patients enrolled in this protocol. Our goals in this clinical trial are to examine its safety and immune responses associated with the peptide vaccination. This clinical trail is considered to be novel in; 1) synchronously injecting wild type and mutant gp100 peptides, and 2) using a newly mapped gp100 peptide restricted to HLA-A*2402. We have enrolled 6 patients for HLA-A*2402 peptide and 5 patients for HLA-A*0201 peptides during year 2002. The protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted. Grade I of skin reaction in the vaccinated site was observed in some patients. However, Vitiligo was observed in two patients with HLA-A24. In one patient with HLA-A24, there was obvious decrease of the liver metastasis. Based on these results, we have prepared clinical phase II study using HLA-A24 restricted gp100 derived peptide.

3. Development of detection method of minimal residual disease for gastrointestinal cancer

Naomi Kimura, Takuya Tsunoda, Kenichi Ohyama, Hiroaki Tanaka, Shogo Nakano, Takuya Takayama, Yoshifumi Beck, Hideaki Tahara

Hematogenous metastasis is one of the most frequent causes of treatment failure following gastrointestinal cancer surgery. In these cancer patients, dormant minimal residual disease (MRD) is often asymptomatic and clinically undetectable until relapse. Thus, detection of MRD is needed to improve the treatment of gastrointestinal cancer, and may provide useful information for selecting candidates for adjuvant chemotherapy. Recent molecular biological evidence has demonstrated that microscopic cancer cells are detectable by immunohistological staining and RT-PCR. In this study, immunohistological staining is assayed using anti cytokeratin antibody and anti CEA antibody in bone marrow aspirates and primary tumors. Furthermore, real-time RT-PCR assay is performed for measuring CEA, CK-19 and CK-20 mRNA in bone marrow aspirates and peripheral blood and primary tumors. Bone marrow aspirations are obtained from sternum after operation. Peripheral blood samples are obtained from femoral artery before and after operation. A total of 16 gastrointestinal cancer patients (8 gastric cancer and 8 colorectal cancer) who undergo curative operation are enrolled in this study. The present study investigates the feasibility and clinical utility of this procedure for sensitive detection of hematogenous metastasis in gastrointestinal cancer patients. We have successfully established the probes from CEA, CK-19 and CK-20, and evaluated the cut-off lines of each target molecules

4. Development of Immunotherapy Using Dendritic Cells in Combination with Local Radiation Therapy and Systemic Administration of IL-2

Takuya Takayama, Hiroaki Tanaka, Juichiro Konishi, Naomi Kimura, Takuya Tsunoda, Yoshifumi Beck, Hideaki Tahara

We have already reported that intra-tumoral administration of dendritic cells (DC) induced anti-tumor response in several pre-clinical models. From these background, we have started to recruit the patients with metastatic tumor in the skin as a phase I clinical trial and treated them using intra-tumor administration of dendritic cells in combination with local irradiation and systemic administration of interleukin-2. The DCs are generated by 8 days incubation with granulocyte stimulating factor and interleukin-4 of peripheral blood monocytes obtained by leukapheresis. Before intra-tumoral administration of DC, tumor in the skin is irradiated. After administration of DC, interleukin-2 is injected subcutaneouly. This phase I clinical trial was initiated to test the toxicity and biological effects in this protocol. We have enrolled 7 patients and safely finished the treatment of 5 patients and observed no clinical side effects as of the end of December in 2002.

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Our department consists of three major divisions: diagnostic radiology, nuclear medicine and radiation oncology. Diagnostic radiology plays a critical role in evaluating various neoplastic and infectious diseases. Clinical studies are conducted mainly using magnetic resonance imaging, supported by other departments and other institutions. In nuclear medicine, we develop analytic methods to estimate in vivo physiology, as well as studying the kinetics of radiotracers and physical characteristics of detectors. In radiation oncology, total body irradiation prior to bone marrow transplantation provides valuable advantage.

1. Phantom and Animal Studies of a New Hepatobiliary Agent for MR Imaging, Gd-DTPA-DeA: Comparison with Gd-EOB-DTPA

Kohki Yoshikawa, Yusuke Inoue, Masaaki Akahane, Morio Shimada[1], Sayaka Itoh[2], Atsushi Seno[2], and Sanshin Hayashi[1]: [1]Department of First Radiology, Toho University; [2]Department of Radiology, Tokyo Metropolitan University of Health Sciences

The aim of this study was to investigate the characteristics of Gd-DTPA-DeA as a hepatobiliary contrast agent for MR imaging in comparison with Gd-EOB-DTPA. Phantom experiments were undertaken to assess T1 relaxation times and signal intensities on SPGR images for Gd-DTPA-DeA, Gd-EOB-DTPA, and Gd-DTPA in human plasma. For Gd-DTPA-DeA and Gd-EOB-DTPA, contrast effect was evaluated in the rats using a SPGR sequence. The contrast ratios of the liver and abdominal were measured up to 21 minutes after intravenous administration. Visualization of the bile duct and renal pelvis was also assessed. In human plasma, T1 relaxation times were similar for Gd-DTPA-DeA and Gd-EOB-DTPA and shorter than for Gd-DTPA. Whereas the contrast ratio of the liver reached the peak at about 5 minutes after the injection of Gd-EOB-DTPA with a subsequent fall, continuous rise was shown for Gd-DTPA-DeA, resulting in larger maximal contrast effect. Contrast ratios of the abdominal aorta was larger for Gd-DTPA-DeA. Biliary excretion was observed for both agents and was earlier for Gd-EOB-DTPA. While renal excretion was shown for all rats 3 minutes after the injection of Gd-EOB-DTPA, it was not observed for Gd-DTPA-DeA. Gd-DTPA-DeA may be used as a hepatobiliary contrast agent and shows different pharmacokinetics from Gd-EOB-DTPA.

2. Evaluation of quantified proton magnetic resonance spectroscopy (^{1}H-MRS) of cerebral disorders using automated three-dimensional (3D) tissue segmentation

Kohki Yoshikawa, Yusuke Inoue, Takehiro Yoshikawa, Seizo Takahashi[3], and Takashi Ogino[4]: [1]Department of Chemical & Biological Sciences, Japan Women's University; and [2]National Institute of Neuroscience

The aim of this study is to evaluate our developed techniques for automated 3D tissue segmentation

and for quantified ^{1}H-MRS of cerebral disorders. We used two phantoms for validation of accuracy of our techniques. One of them was the phantom with various contents of animal hide and agar, which resembled normal gray matter, white matter, cerebral tumor, or surrounding edema. Another phantom was composed of various contents of cerebral metabolites such as N-acetylasperate, choline, creatine, glutamate, glutamine, lactate, myo-inositol and so on at 7. 0 – 7. 1 pH. The images used in our automated 3D tissue segmentation were 3D SPGR images with slice thickness of 1mm and 2D T2 weighted and proton density weighted images with slice thickness/slice gap of 2mm/0mm. ^{1}H-MRS was performed by point-resolved spectroscopy (PRESS) sequence at 1. 5-Tesla clinical MR scanner. The accuracy of 3D tissue segmentation technique was quite high enough for using quantified ^{1}H-MRS, but variation was seen at the result of quantification of each metabolites. Improvement of compensation methods for gradient field, RF field, and relaxation time of each metabolites should be necessary for its clinical use. This quantified ^{1}H-MRS using automated three-dimensional tissue segmentation will enable to provide accurate higher resolutional metabolic information of cerebral disorders in short processing time and will play an important role for diagnosing cerebral disorders, planning treatment methods, and evaluating treatment effect.

3. Effect of chollimator characteristics on scintigraphic assessment of cardiac sympathetic nervous system

Yusuke Inoue, Ichiro Shirouzu[5], and Toru Machida[5]: [5]Departments of Radiology, Kanto Medical Center NTT EC

Quantitative accuracy in ^{123}I studies may be impaired by septal penetration. We evaluated the effect of collimator choice on estimation of the heart-to-mediastinum (H/M) ratio in cardiac ^{123}I-MIBG imaging. Three collimators were used: a low-energy high-resolution (LEHR) collimator (septal penetration at 159 KeV, 4. 83%), special LEHR (SLEHR) collimator (0. 93%), and medium-energy (ME) collimator (0. 00%). A nine-cell phantom (termed a checker phantom), whose cells were filled with various concentrations of ^{99m}Tc or ^{123}I solution, was imaged with the three collimators to assess contrast accuracy. Using a thoracic phantom containing ^{123}I solution, we examined the effects of lung and liver activities on heart and mediastinum counts. In eight patients, anterior chest views were acquired successively with the three collimators about 3. 5 hours after ^{123}I-MIBG injection, and heart-to-mediastinum (H/M) ratios were compared between collimators. The validity of scatter correction by the triple-energy-window (TEW) method was also examined in phantom experiments. In the checker phantom studies, the use of the LEHR collimator caused overestimation for cells containing low ^{123}I activity. Overestimation was less with the SLEHR collimator and declined further with the ME collimator. Contrast accuracy in imaging ^{123}I sources with the ME collimator was comparable to that for ^{99m}Tc. Thoracic phantom studies demonstrated contamination of heart and mediastinum counts by lung and liver activities. Contamination was greatest with the LEHR collimator and least with the ME collimator. H/M ratios in the patients were significantly higher with the SLEHR collimator (2. 04 ± 0. 48) than with the LEHR collimator (1. 81 ± 0. 29, $p < 0.05$), and were still higher with the ME collimator (2. 66 ± 0. 74, $p < 0.05$). The difference in H/M ratios between the LEHR and ME collimators showed a high positive correlation with the lung-to-mediastinum ratio ($p < 0.0001$) but was not correlated with the liver-to-mediastinum ratio. Scatter correction tended to improve quantitative accuracy but failed to provide consistently successful results. In conclusion, collimator choice substantially influences estimation of the H/M ratio in cardiac ^{123}I-MIBG imaging. The use of an ME collimator eliminates the degradation of quantitative accuracy caused by septal penetration and may enhance reliability in the evaluation of cardiac sympathetic nerve function. Now, we are going forward to single photon emission tomography (SPECT), with the assumption that the higher spatial resolution provided by the SLEHR collimator may be critical in SPECT imaging.

4. Quantitative evaluation of skeletal muscle glucose utilization by ^{18}F-FDG PET

Yusuke Inoue and Ikuo Yokoyama[6]: [6]Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo

PET with [^{18}F]2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) can be used for quantifying skeletal muscle glucose utilization (SMGU) to characterize insulin resistance. We measured SMGU of the femoral region using ^{18}F-FDG PET, and examined the effects of two technical alterations, shortening the imaging period for analysis and partially replacing arterial blood sampling by venous sampling, on the estimated value. Ten patients with coronary risk factors were included in the analysis. Dynamic ^{18}F-FDG PET imaging of the femoral region was performed under insulin clamping, accompanied by frequent arterial and venous blood sampling. SMGU was determined by graphical analysis based on the application of a three-compartment model and neglection of dephosphorylation of ^{18}F-FDG-6-phosphate. SMGU-A7, SMGU-A4 and SMGU-A3 were calculated using the last seven (10. 75-60. 75 min after tracer injection), four (25. 75-60. 75 min) and three (30. 75-60. 75 min)

frames of PET imaging, respectively, combined with the input function derived from arterial blood sampling. SMGU-V7 was defined by partially substituting arterial plasma activity by venous plasma activity in calculating SMGU-A7, and SMGU-V4 and SMGU-V3 were similarly computed. SMGU-A7 was 43. 8 ± 29. 2 μmol/min/kg, ranging from 6. 5-85. 2 μmol/min/kg. SMGU-A4, SMGU-A3, SMGU-V7, SMGU-V4 and SMGU-V3 had almost the same mean value to SMGU-A7, and were closely correlated to SMGU-A7. Imaging period used for analysis had limited effect on the estimates of SMGU, suggesting the validity of measuring myocardial glucose utilization and femoral SMGU by using a single injection of ^{18}F-FDG and early chest imaging followed by femoral imaging. Omission of arterial blood sampling may be justified in such combined measurement. In addition, we studied simple quantification of SMGU by static PET imaging. Standardized uptake value (SUV) was calculated at 45 min and 55 min after tracer injection. Skeletal muscle to background ratio (SM/B ratio), tissue count divided by venous plasma activity, was also computed at 45 min and 55 min. These simple indices were compared with measured SMGU by linear regression, and SMGU was estimated using the obtained regression equation and simple index. SMGU was highly correlated with SUVs (r = 0. 941 at 45 min, r = 0. 951 at 55 min) and SM/B ratios (r = 0. 968 at 45 min, r = 0. 984 at 55 min). Although SMGU was almost proportional to SM/B ratios, the y-intercepts of the regression lines for SUVs significantly differed from zero. The residual in estimating SMGU using the regression equation was marginally smaller for SM/B ratios than for SUVs and for indices at 55 min than for 45 min, with no statistical significance. Correction for plasma glucose level slightly elevated correlation coefficients between SMGU and simple indices. In conclusion, simple quantitative indices, SUV and SM/B ratio, are suggested to be reliable indicators of SMGU during hyperinsulinemic euglycemic clamping. Static imaging with or without single venous blood sampling may replace dynamic imaging with frequent arterial blood sampling, offering substantial convenience in evaluating insulin resistance.

5. Assessment of renal parenchymal damage after nephron-sparing surgery

Yusuke Inoue and Shigeharu Kurimoto[7]: [7]Department of Urology, Graduate School of Medicine, University of Tokyo

Nephron-sparing surgery is a treatment in which a part of a diseased kidney is resected and some parenchyma of the kidney is spared. Although the advantage of the technique resides in its ability to preserve renal function, surgical intervention may damage the spared renal parenchyma. Prolongation of renal parenchymal retention of ^{99m}Tc-MAG3 has been demonstrated in acute renal damage such as acute tubular necrosis. The aim of this study was to determine whether or not parenchymal retention of ^{99m}Tc-MAG3 is prolonged after nephron-sparing surgery. Twenty-two patients underwent a total of 29 ^{99m}Tc-MAG3 renal scintigraphic studies within one year after nephron-sparing surgery. In 17 patients (23 examinations) who had bilateral kidneys, parenchymal retention in the operated kidney was compared visually with that in the contralateral kidney, and the presence of diffuse prolongation of parenchymal retention was determined. In all patients, regional parenchymal retention was compared within the treated kidney, and the presence of regional prolongation around the surgical margin was assessed. Diffuse prolongation of parenchymal retention was observed in four of 10 examinations performed within one month after surgery and in none of 13 examinations performed later than one month after surgery. Regional prolongation was shown in 10 of 14 examinations performed within one month after surgery and in three of 15 examinations performed later than one month after surgery. In five patients who were studied both prior to and later than one month after surgery, regional prolongation around the surgical margin was noted on the first study. On the second study, regional prolongation was improved and initial renal uptake around the surgical margin was intensified. In coclusion, Renal parenchymal retention of ^{99m}Tc-MAG3 may be prolonged in the early period after nephron-sparing surgery. Renal scintigraphy with ^{99m}Tc-MAG3 appears to aid in characterizing acute renal damage, as well as in estimating preserved renal function, after nephron-sparing surgery.

6. Diffusion tensor imaging of cerebral infarction: analysis of ADC and DTI scalar metrics (fractional anisotropy and eigenvalues)

Takeharu Yoshikawa and Shigeki Aoki[8]: [8]Department of Radiolody, University of Tokyo

The aim of this study is to describe changes of diffusion tensor (DT) scalar anisotropy metrics (fractional anisotropy (FA) and eigenvalues) in acute and subacute infarction and to explore the usage of DT images including 3D tractography in stroke patients. Thirty-seven patients with acute and subacute infarction within 14 days from onset were studied by 1. 5T MR imagers using diffusion tensor echo planar imaging with 6 or more different motion probing gradient directions. Distortion correction and analysis of DTI scalar metrics (FA and eigenvalues) were performed on a workstation (AW; GE). DT 3D tractography using seed and track method was reconstructed by home-built software on PC. ROIs were located on the infarcted areas which showed

hyperintensity on diffusion-weighted images and were confirmed by follow-up MR and/or CT. White matter (WM) and gray matter (GM) were separated using FA images and located separately. Infarction in the WM and GM showed different changes in DT scalar metrics (average FA: 0. 175 SD 0. 071 in GM vs 0. 323 SD 0. 142 in WM). In both WM and GM infarct with ADC more than 5. 5 x 10^{-4} mm^2 /s, significant negative correlation was observed between ADC and FA. In the white matter with ADC more than 5. 5 x 10^{-4} mm^2 /s, parallel positive correlation was observed between each eigenvalues versus ADC. A simulation model, which add or subtract the same constant value on/from each eigenvalues, correlated well with the results above, indicating that effect of vasogenic edema dominate changes of FA when ADC is more than 5. 5 x 10^{-4} mm^2 /s. At the lesions with ADC less than 5. 5 x 10^{-4} mm^2 /s, heterogeneity was observed. DT tractography was useful to clarify relationship between lesions and the white matter tracts especially the pyramidal tract. FA depends on ADC when ADC is more than 5. 5 x 10^{-4} mm^2 /s in infarcted areas, and in such a situation FA changes may not indicate 'anisotropic' changes. Attention should be paid in analysis using FA when ADC is relatively high.

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Our department consists of five subdivisions of clinical physiology, hematology, biochemistry, bacteriology and pathology, and engages in laboratory analysis and diagnosis of clinical materials submitted from the Research Hospital. Along with the ongoing practice of translational research projects in the hospital, we are now under radical reconstruction process to evolve into function as an integrated diagnosis & monitoring laboratory.

General Scheme

Our basic research strategy is to characterize molecular mechanisms underlying pathology, develop a way to measure this in the clinical materials or in human disease *in situ*. To conduct the ongoing clinical trials in an evidence-based way and to promote translational research, our research direction is to develop molecular /surrogate endopoints and assess these biologic makers. Developing molecular endpoint assays in clinical materials requires expertise in pathology and molecular biology, we are thus focusing our specialty to achieve this goal.

1. Comprehensive hematological analysis

Our (N. Oyaizu & K. Takeuchi) specialty is pathology and both have expertise in analyzing immuno-hematological disorder including leukemia/lymphoma and transplantation-associated disorders. Not only elucidating the pathological mechanisms by traditional morphological approaches, we are now constructing infrastructure which enables us to conduct integrated (molecular-based as well as functional) analysis thereby pursuing comprehensive pathological research especially focusing on immuno-hematological disorders.

2. Pathological evaluation of cancer immunotherapy

We have initiated to analyze the surgical materials obtained from patients under cancer immuno-therapy conducted in the research hospital. By applying sophisticated immunohistochemical techniques, we are now intensively analyzing materials from cases including GM-CSF-based gene therapy for renal cell carcinoma and dendritic cell-based or peptide-pulsed anti-melanoma immuno-therapy. Our goal is to evaluate the effectiveness and to elucidate the mechanisms of anti-tumor immune response elicited by the therapy *in situ*.

3. Elucidation of immunopathological mechanisms of autoimmune-based hematological disorders

Among the patients with hematological disorder such as myelodysplastic syndrome-refractory anemia (MDS-RA), aplastic anemia, or pure red cell aplasia, we noticed common patho-immunological mechanisms are operative under these hematological abnormalities. That is destruction of erythroid precursors by immune-based mechanisms in the

bone marrow. Our goal is to elucidate molecular mechanisms on the ground of pathology thereby develop new therapeutic interventions.

4. Developing new immunological laboratory methods to predict and monitor Graft versus. Host Disease (GVHD)

GVHD is a life-threatening immunological disorder associated with bone marrow transplantation. Diagnosis of GVHD is now solely depending on pathological examination of biopsy specimen, and there is no reliable laboratory indicator predicting the onset of GVHD. We are planning to establish new immunological methods by examining gene expression profile *in situ* thereby pinpointing the molecular markers specifically expressed in GVHD.

5. Developing quick & inclusive diagnosis system for infections disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions altered drastically and came into aspects. For example, host received allogeneic BMT resulted in chimeric state composed of donor-derived immune system while host somatic cells processes own MHC molecules. This results in altered recognition and molecular interaction of infected cells with immune cells, which leads to atypical pathological as well as clinical manifestations. To distinguish infectious disease and immunological disorder is critical issue, however as a result of modified manifestations, in some occasion, it is a difficult to achieve. To circumvent this issue, we are pursuing to establish quick and inclusive diagnosis system of infectious disease.

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Research Hospital

Department of Applied Genomics
ゲノム診療部

Associate Professor	Noriharu Sato, M.D., D.M.Sc.	助教授	医学博士　佐藤典治
Clinical Associate	Naoyuki Takahashi, M.D., D.M.Sc.	助手	医学博士　高橋直之

Our department was established in April, 2001 to support the translational researches of our hospital. Ongoing projects which we are participating in are immunogene therapy of neuroblastoma and immune therapy for thyroid cancer utilizing dendritic cells immunized with tumor antigens. We are also conducting researches to find responsible SNPs in genes related to drug sensitivity, disease progression, and prognosis in various hematological disorders. In December, 2002 we opened a clinic of genetic counseling in collaboration with the divisions of pediatrics, genetic diagnosis, nursing and so on.

1. Genetic study on CML

Naoyuki Takahashi and Noriharu Sato

Before the advent of STI571, interferon has been the first choice drug for patients with CML who had no HLA-identical sibling donors. Since the long-term effect of STI is still unclear, interferon may have some position in the treatment of CML. We are going to determine SNPs in genes related to the sensitivity of interferon in patients with CML.

2. Genetic study on MDS

Naoyuki Takahashi and Noriharu Sato

Myelodysplastic syndrome (MDS) is heterogeneous diseases with different prognosis and different drug sensitivities. Some MDS respond to steroid therapy and some do so to cytokine therapies. We are studying on the SNPs in genes related to cytokine signaling or apoptosis whether there is some association between these SNPs and drug sensitivities or disease prognosis. Immature stem cells from MDS may have different expression profiles from that of normal individuals. We are now preparing oligonucleotide arrays to examine the hypothesis.

3. Genetic study on GVHD

Naoyuki Takahashi and Noriharu Sato

In hematopoietic stem cell transplantation, we sometimes observe severe GVHD (graft-versus-host disease) in HLA-matched transplant. There are reports suggesting an important role of cytokines and non-classical HLA molecules in GVHDs. In fact, promoter polymorphism of TNF gene is reported to be involved in severe GVHD. In order to find other genes affecting the severity of GVHD in addition to TNF, we are studying SNPs in genes related to immunologic responses.

Publications

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Research Hospital

Division of Clinical Trial Safety Management
医療安全管理部

Professor	Naohide Yamashita, M.D., Ph.D.	教　授　医学博士	山　下　直　秀
Clinical Associate	Fumitaka Nagamura, M.D., Ph.D.	助　手　医学博士	長　村　文　孝

Division of Clinical Trial Safety Management (DCTSM) was established on April 2001 in the Research Hospital to watch the safety and ethics of clinical trials. DCTSM also deals with the risk management of diagnosis and treatment for general medicine. The staffs of DCTSM (doctors and nurses) are doing their work in collaboration with Translational Research Coordinators (TRC), which are organized by co-medical staffs, including pharmacist, dietician, psychologist and clinical laboratory technologist. The aim of DCTSM is to carry out the safe and ethically-protected clinical trials in the Research Hospital in addition to the data management. In order to accomplish it we are doing the following activities.

1. Review of clinical study protocols before the review of Institutional Review Board (IRB: Chiken-Sinsa-linkai)

Fumitaka Nagamura and Naohide Yamashita.

One of the roles of our division is to keep the quality of protocols. To perform this task, we discuss and advise on a protocol with principal investigators, and made it a rule to submit a protocol and written consent form before submitting to the Institutional Review Board.

From January 2002 to December 2002, we received six protocols and numerous questions. Five protocols were submitted from Department of Surgery. All the protocols were Phase I/IIa studies on peptides with IL-2 for patients with malignancies. The other one was submitted from Department of Infectious Disease and Applied Immunity. In this study, pulsed dendritic cells will administered for patients with HIV. Pre-review of these protocols were finished within two to three weeks from the receipt. The format of pre-review is based on the style of applied in the U. S. Food and Drug Administration. Our opinion is summarized into three sections: safety issue (most concern), major problem, and minor problems/suggestions. These opinions are not obligations to have enforcement, but those to improve clinical studies. Final decision should be made at the Institutional Review Boards.

To assist the planning of clinical studies and writing protocols, we disclosed "Guideline". New Good Clinical Practice and Guidelines of International Conference on Harmonization has been settled, and many guidelines for gene therapy and gene analysis have been announced. Our division prepared these guidelines, and tries that intra-institutional clinical studies respect such guidelines.

2. Studies on approval of oncologic drugs.

Fumitaka Nagamura

Evaluation of drugs and theraputic methods should be made scientifically. One goal of them is to be approved by the administrative office. The U. S. Food and Drug Administration (FDA) is the administrative office in the U. S., not in Japan. However, one of its aims is to study the drug approval system and methodology. In terms of these respects, FDA is the most advanced institution in the world.

We cooperated with Division of Oncology Drug Products of Center for Drug Evaluation and Research of FDA to study above things. At the annual meeting of American Society of Clinical Oncology, we presented the results of our works. One was the evaluation of dose escalation schemes of Phase I oncologic drugs. We revealed that two novel methods, pharmacologically guided dose escalation and modified continual reasessment method, have not shown the advantages on the classical modified Fibonacci method. The other one was the construction of electric database on approved procedures of oncologic drugs in the U. S. By using this database, we will reveal the results of studies, lesions contributed to pivotal studies, role of QOL on approval and problems of Subpart H (accelerated approval) at the next meeting.

Although these studies related to the U. S. regulations, not those of Japan, it is useful to have the knowledge on drug approval systems. The reason why these studies are useful is that all the therapeutic methods, including translation researches, should be considered their situations. Almost all of the eligible patients for translational research would be the refractory/failed to standard therapy. In Japan, the concept has not been settled. We have to evaluate the standard therapy in Japan at almost all diseases. However, our division can have the world-wide information by this study. The results have been applied at the candidate evaluation in Translational Research.

3. Monitoring at the completion of study

Fumitaka Nagamura and Naohide Yamashita

Monitoring and audit for clinical studies are inevitable to ascertain that clinical studies have been performed ethically and scientifically. Generally, monitoring is the routine check-up and audit is done when the suspicion of fraud for a clinical study exists. We take place the weekly TRC meeting. We can resolve the problems on clinical trials at the meeting immediately, because principal investigators and co-investigators attend the meeting. This fact is the great help to maintain the quality of studies.

Monitoring is usually performed annually and at the time after the completion of the study. Our division performed the monitoring after the completion of study for "Gene therapy using GM-CSF gene for patients with advanced renal cell carcinoma". This is the first gene therapy for oncologic disease in Japan. Through the monitoring, we did not find no critical problems. Intra-institutional monitoring and that from an organization apart from the institute will be required. We have prepared to perform intra-institutional monitoring, and cooperation with other organization to perform the inter-institutional monitoring should be needed.

4. Education program for Translational Research Coordinator

Fumitaka Nagamura, Hajime Kotaki and Naohide Yamashita.

The major purposes of Translational Research Coordinator are to keep patients' right, to conduct translational research more ethically, and to perform translational research scientifically. The role of TRC is not the same as that of Clinical Research Coordinator CRC) in terms of the aggressive intervention to keep studies ethically conducted. The problem of education for research coordinators including CRC is the new but the critical in Japan. To educate TRCs, we take place seminars after the weekly TRC meeting.

However, there was no good text in Japan. So, we prepared textbooks by ourselves. One textbook is the one used at the CRC educational program held at the U. S. Dr. N. Yamashita joined the Society and attended the meeting. We translated it into Japanese and modified to meet the Japanese environment. Almost all of TRCs attended the seminar based on this, and mastered the content. Another textbook is the one that we have been writing originally. The content is the following: accidents happened during the development of clinical researches; lows and regulations related to clinical studies; what is the clinical research; the reason why clinical studies are necessary; classification of studies, and so on. The latter one has not been completed yet. Seminars based on these textbooks will be planned regularly.

To collect information and to keep up with the development of medicine and environment of clinical studies, we attended the educational programs and lectures. These results were reflected into the education for TRCs. We try to settle the education system for TRC. After the completion of this plan, IMS/UT will be the base for education as well as the one for conducting translational research.

Risk management of Research Hospital

Naohide Yamashita, Fumitaka Nagamura, Yuko Ogami

To perform clinical trials, it is indispensable to assure the safety of daily medical treatments and accuracy of diagnosis. Staffs of DCTSM also engage in the risk management in the Research Hospital. Medical accidents and incidents are reported to DCTSM by written forms. When the urgent response to the patient is required, the meeting is immediately held to discuss the first lines of action to protect the involved patient. This meeting also determines the preventive measures. This kind of meeting was held 18 times in this year. Medical accidents and the responses of DCTM are reported in the Council of Risk Management in the Research Hospital, which is held every three months.

Educational seminars on risk management are required to avoid the medical accident. DCTSM and Nursing Quarters took place two seminars this year. One was the lecture by an airplane pilot regarding to the risk management at the aircraft company, JAL. The other was the role-playing act based on the medical accidents actually happened. After reviewed the act, staffs at the Research Hospital discussed on the cause and prevention. Through these educations, consciousness for risk management will be tightened.

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Research Hospital
Department of Transfusion Medicine
輸血部

Professor	Tsuneo A. Takahashi, D. Sc.	教　授　理学博士	高　橋　恒　夫
Lecturer	Tohru Iseki, M. D., Ph. D.	講　師　医学博士	井　関　　徹

Improving the clinical outcome of hematopoietic stem cell transplantation, we have been engaged in researches to clarify the cytokine network acting on normal hematopoiesis, and to establish the ex vivo expansion system of hematopoietic stem cells. For the successful engraftment of cord blood cells, we are engaging in the basic researches to establish novel culture systems for progenitor B cells, and to clarify the mechanisms how transplanted stem cells are homing to the bone marrow environment. For the future development of regenerative medicine, we also study the differentiation processes of mesenchymal stem cells to various types of cells and/or tissues.

As the clinically-based department, we supply purified hematopoietic stem cells to clinical trials for allogeneic bone marrow and peripheral blood transplantation. The depletion of T lymphocytes from the donor cells is undertaken to reduce the rate of graft-versus-host disease, and the purification of CD34$^+$ cells is performed in order to purge grafts of tumor cells. These clinical-oriented researches are focused not only on cytokine therapy and immunotherapy, but also on gene therapy, antisense therapy, and cell therapy that are being generated in the Institute.

1. Purification of human hematopoietic progenitor and stem cells for bone marrow, peripheral blood transplantations in the clinical setting

Tohru Iseki, Taira Maekawa, Kazuo Ogami, Yuka Wada, Shinobu Hosoda, Tsuneo A. Takahashi[1], Tokiko Nagamura[1], Jun Ooi[2], Akira Tomonari[2], Kaoru Uchimaru[2], Fumitaka Nagamura[2], Atsushi Manabe[3], Kouichiroh Tsuji[3], Arinobu Tojo, and Shigetaka Asano[2]: Departments of [1]Cell Processing, [2]Hematology-Oncology, and 3P[e]diatrics, The Institute of Medical Science, The University of Tokyo

Cell surface antigen CD34$^+$ cells contain the majority of human hematopoietic progenitors and stem cells, that can produce a variety of hemopoietic colonies and reconstitute the hematopoiesis after myeloablative chemotherapy. Several methods to purify a large number of CD34$^+$ cells from the bone marrow (BM) and peripheral blood (PB) samples have been developed such as the panning and the culumn filtration methods with immunobeads. The purity and recovery efficiencies after separation using immunobeads in our department are more than 98% and 50-60%, respectively. The administration of more than 5×10^5/kg CD34$^+$ cells purified from bone marrow and 2×10^6/kg CD34$^+$ cells purified from PB after mobilization by granulocyte colony-stimulating factor (G-CSF) are capable of inducing a rapid

and permanent recovery of the hematopoiesis after transplantation. Allogeneic peripheral blood stem cells have now been used as an alternative method for clinical transplantation (allo-PBSCT). In order to obtain less graft versus host disease (GVHD), bone marrow and peripheral blood transplantations using allograft of purified $CD34^+$ cells are now used in the clinical settings.

2. Establishment of enzyme linked immunosorbent assay to detect soluble HLA class I antigens in serum from patients received allogeneic stem cell transplantations

Tohru Iseki, Taira Maekawa, Kazuo Ogami, Yuka Wada, Shinobu Hosoda, Tsuneo A. Takahashi[1], Tokiko Nagamura[1], Kouichiroh Tsuji[3], Arinobu Tojo, and Shigetaka Asano[2]

Besides being expressed on the membrane of most nucleated cells, HLA class I antigens are present in serum. We established the emzyme linked immunosorbent assay to detect soluble HLA class I antigens in serum. An increase in the serum HLA class I antigen level has been seen in acute rejection episodes following heart, liver, and kidney transplants. We found that soluble HLA class I level significantly increases in patients suffering from acute graft versus host disease (GVHD) episodes following allogeneic bone marrow transplantation (allo-BMT) whereas it does not change in patients without GVHD. We are now investigating whether the increase of this soluble HLA Class I antigen levels in serum from patients received allo-BMT, -PBSCT, and cord blood transplantation (CBT) can modulate the immunoregulatory systems leading to less onsets of GVHD, comparing with other cytokine levels including interleukins, interferons, and tumor necrosis factor.

3. Megakaryopoiesis of cord blood cells are effectively enhanced by stromal cells derived from bone marrow mesenchymal stem cells

Tohru Iseki, Taira Maekawa, Kazuo Ogami, Yuka Wada, Shinobu Hosoda, Tsuneo A. Takahashi[1], Tokiko Nagamura[1], Fumitaka Nagamura[2], Kouichiroh Tsuji[3], Arinobu Tojo, and Shigetaka Asano[2]

Mesenchymal stem cells (MSCs) give rise to marrow stromal cells that produce the spongy stromal matrix comprising the bone marrow microenvironment. These marrow stromal cells contribute directly to blood cell formation by producing the extracellular matrix where blood cell development takes place and by providing cytokines and other molecules that direct or stimulate the production of mature blood cells. MSCs are rarely contained in cord blood, that may cause the delayed engraftment of megakaryopoiesis in a clinical setting of cord blood transplantation. Preclinical studies in animals have demonstrated that culture-expanded mesenchymal stem cells can be used to repair bone defects, full-thickness articular cartilage defects, bone marrow stroma, and tendon. We are now seeking to develop human therapeutic products based on the role of hMSCs in megakaryocytopoiesis. We found that stromal cells derived from MSCs effectively suuport the megakaryocytopiesis of cord blood cells *in vitro*.

5. Establishment of Room for Clinical Cellular Technology (RCCT)

Tohru Iseki, Taira Maekawa, Kazuo Ogami, Yuka Wada, Shinobu Hosoda, Tsuneo A. Takahashi[1], Tokiko Nagamura[1], Kaoru Uchimaru[2], Kouichiroh Tsuji[3], Arinobu Tojo, and Shigetaka Asano[2]: Departments of [1]Cell Processing, [2]Hematology-Oncology, and [3]Pediatrics, The Institute of Medical Science, The University of Tokyo

Cell therapy including stem cell transplantation and gene therapy being ultimate therapeutic approaches for incurable diseases, their establishments are urgently needed. It is also mandatory to separate and manipulate cells under quality-controlled sterilized circumstances that can meet with GMP approvals, and provide powerfully enegineered cells to clinical settings. For this purpose, the center having clean rooms (Room for Clinical Cellular Technology:RCCT) with clinical P2 and P3 facilities is now operating in the Institute. The banking of cord blood cells for cord blood transplantation, the insertion of GM-CSF gene to renal tumor cells using retrovirus vector for gene therapy, and the generation of antigen-pulsed denderitic cells against malignant melanoma cells are on going in RCCT.

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Research Hospital
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Associate Professor Masakazu Hayashida
Clinical Associate Shin-ichi Watanabe

助教授 林 田 真 和
助 手 渡 邉 慎 一

Our clinical practice and clinical as well as experimental studies have been focused on (1) anesthetic management in patients undergoing major cardiovascular surgery, (2) management of intraoperative and postoperative pain, and (3) management of chronic intractable pain. We have published several works on these subjects last year.

1. Anesthetic management in patients undergoing major cardiovascular surgery especially focusing on monitoring of cerebral oxygenation and cerebral function

The Bispectral Index (BIS) is a recently developed derivative of processed electroencephalogram that has been proven to closely correlate with level of consciousness during natural sleep and general anesthesia. It has been widely used in the area of anesthesia to evaluate sedative/hypnotic state in patients undergoing surgery under general anesthesia.

We have found that BIS is also useful to trace suppression and recovery of cerebral electrical activity in patients undergoing surgery of the thoracic aorta using deep hypothermia and circulatory arrest (DHCA) 1). In patients subjected to long-lasting DHCA, recovery of BIS was delayed substantially.

We have also found that BIS is also useful to detect cerebral ischemia during pediatric and adult cardiac surgery especially when used in combination with the near-infrared spectroscopy (NIRS) to measure oxygen saturation of the brain 2)3). Simultaneous monitoring with BIS and NIRS revealed that in children, especially in infants, cerebral ischemia seemed to occur frequently during cardiac surgery presumably due to immaturity of the cerebral vascular autoregulation 2)3).

We also reported successful anesthetic management of critically ill patients 4)5).

2. Management of intraoperative and postoperative pain

We have published several works on management of intraoperative and postoperative pain 6)-16). We have developed a rabbit model of surgical anesthesia/analgesia, which allows for repeated and quantitative evaluation of depth of surgical anesthesia/analgesia provided by a variety of anesthetics/analgesics 6)-9). We also published several review articles on how to manage postoperative pain 10)-12), and original articles comparing various modalities of postoperative pain management 13)14)15). By comparing respiratory effects of epidural opioids, we have found that mu-receptor agonists and not kappa-receptor agonists are effective in improving respiratory function after upper abdominal surgery when administrated epidurally, and have concluded that mu-opioids are preferable to kappa-opioids as epidural analgesics for postoperative pain management 16). We also reported successful management of prolonged post-dural puncture headache, one of major complications of attempted epidural analgesia, with a dual blood patch technique 17).

3. Management of chronic intractable pain

We published several works on new treatment modalities for chronic intractable pain syndrome in-

cluding ketamine capsule, clonidine ointment, and continuous intravenous infusion of adenosine or ATP 18)-20). We have also evaluated the analgesic effect of intravenous morphine on a variety of chronic pain including neurogenic pain, post-herpetic pain, prolonged post-traumatic or post-surgical pain, intractable low back pain, and so on, and have found that morphine is more or less effective in controlling all kinds of chronic pain 21). We also investigated the effect of a circadian rhythm on a frequently used analyzer of neuronal function 22).

We will continue to research on these subjects and publish several additional reports this year.

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