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Department of Advanced Medical Science was established in September 1997. We are investigating, (1) analysis of the Gradient Expression of Genes in Human Colonic Mucosa, (2) analysis the role of newly identified non-coding RNA for organ development, (3) analysis on the mechanisms of cardiac outflow tract development, (4) serological identification of melanoma antigens by recombinant expression cloning. We are planning and progressing several projects described below to develop new therapies for several diseases, including cancer and ischemic disorders.

Analysis of the Gradient Expression of Genes in Human Colonic Mucosa

Ohno H. et al.

Ulcerative colitis (UC) is characterized by continuous inflammation extending from rectum to oral colonic mucosa. Epidemiological data have provided incontrovertible evidence that both genetic and environmental factors are important in the disease susceptibility. We speculate that the gradient expression of genes in human colonic mucosa might be related to the disease development and progression. In this study, we compared the expression levels of genes in a normal adult human colon and made the catalogue of genes at higher level in the distal colon. First, we compared the expression levels of genes at different segments of colon by screening cDNA microarray. Next, RT-PCR analysis were conducted to confirm the expression levels of these genes. Finally, we evaluated the expression levels of these genes throughout the GI tract and in other tissues by northern blot analysis. As a result of this analysis, the three genes showed the expression gradient to increase toward the distal colon and one of them was specifically expressed in colon. We have generated rabbit polyclonal antibodies against the protein encoded by this gene. In western blot analysis, expression of the protein decreased in many cancer cell lines of colon. We are currently examining whether or not expression of the gene has effect on cellular proliferation.

Analysis of the role of newly identified non-coding RNA for organ development

Watanabe T. et al.

To understand the process of organ development is of importance with respect to the possibility of medical approach for congenital diseases. Recently, Nakaoka et al. identified a novel gene, *Dnm3os*, originally named *Hag2*, which was unlikely to code protein and was expressed tissue-specifically in the mouse development. *Dnm3os*, a gene transcribed into a non-coding

RNA (ncRNA), contains three micro RNAs (miRNAs); miR-199a, miR-199a* and miR-214, whose functions remain entirely unknown in mammals. *Dnm3os* gene expression was developmentally regulated in mouse embryos; initially it was detected at embryonic day (E) 9, became most extensive at E10.5, and decreased from E11.5 thereafter. At E10.5 *Dnm3os* expression was clearly detected in the pharyngeal arches, limb buds and somites. We introduced the lacZ gene into the *Dnm3os* locus to recapitulate its expression pattern and disrupt its function. *Dnm3os*$^{+/lacZ}$ heterozygous embryos showed β-galactosidase activity in the pharyngeal arches, limb buds and somites, just like the authentic expression of *Dnm3os* RNA. *Dnm3os*$^{lacZ/lacZ}$ homozygous mice were born with an expected Mendelian ratio but were small in size. Some *Dnm3os*$^{lacZ/lacZ}$ mice survived postnatally, whereas most homozygous pups developed poorly and died within 1 month. After birth, *Dnm3os*$^{lacZ/lacZ}$ mice exhibited several skeletal abnormalities, including craniofacial hypoplasia with bite overclosure, defects in dorsal neural arches and spinous processes in the cervical and thoracic vertebrae, and osteopenia in vertebrae and long bones. Importantly, the expression of miR-199a, miR-199a* and miR-214 was significantly down-regulated in *Dnm3os*$^{lacZ/lacZ}$ embryos in parallel with *Dnm3os* RNA, supporting the assumption that *Dnm3os* is a precursor of these three miRNAs. Thus, *Dnm3os* has emerged as the first miRNA-encoding gene that has proved indispensable for normal skeletal development and body growth in mammals. Now, the project to investigate the molecular mechanisms responsible for skeletal abnormalities in *Dnm3os*$^{lacZ/lacZ}$ mice is under investigation.

Analysis on the mechanisms of cardiac outflow tract development

Takabe T. et al.

Malformations of the cardiovascular system in the human account for most of the premature deaths caused by congenital abnormalities and, most often, are linked to abnormalities in the formation of the cardiac outflow tract. The heart defect (*hdf*) mouse is a recessive lethal mutation that arose from a LacZ reporter containing a transgene insertional mutation. The most striking feature of the *hdf* homozygous embryo is the immature formation of the outflow tract, through which diminutive right ventricle connects directly to the aortic arches. Therefore, the hdf mouse is a good model system to investigate the formation of the cardiac outflow tract, which will shed light on the molecular mechanisms of congenital abnormalities, especially linked to the outflow tract abnormalities. In this study, suppressive subtractive hybridization revealed decreased mRNA expression of several genes associated with cell survival and normal development of the neural crest cell (NCC) including *Mdk, Cdk4, Skp2, C1d* and *Crabp1* in *hdf/hdf* embryos. Serial sections of whole mounts prepared for in situ hybridization suggested that the initial delamination of NCC from the neural tube epithelium appeared normal in the *hdf/hdf* embryos, however the migration of NCC towards pharyngeal arches was disrupted. Moreover, LysoTracker and TUNEL staining showed that massive apoptosis occurred in the cephalic mesenchyme of *hdf/hdf* embryos. Sinc *Cspg2* locus is disrupted in the heart defect (*hdf*) mouse by transgenic insertion, it was suggested that *Cspg2* might function to promote survival of mesenchymal cells associated with migrating NCC, or function to guide the NCC migration.

Serological identification of melanoma antigens by recombinant expression cloning

Sharif U.A. et al.

We previously conducted dendritic cell therapy on 10 melanoma patients and remarkable tumor reductions were observed in two patients. We aimed to identify some of the unique antigens targeted by dendritic cell therapy in these patients. To identify the target antigens, we first employed two-dimensional electrophoresis combined with Western blots analysis and matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS) methods. Through this strategy, carbonic anhydrase II (CA-II) was identified as an antigen that elicited serum antibody response to dendritic cell therapy in the patient (Yoshiura K, et. al. *Clin. Cancer Res*. 11: 8201-7). To further extend this study, we then employed cDNA expression cloning which is recognized by IgG antibodies in sera from melanoma patient. We constructed cDNA libralies from the melanoma tissues of the patients and the screening is now under way.

Development of immunotherapy using regulatory dendritic cells*

Fujita S, et al. *Collaborated with Laboratory for Dendritic Cell Immunobiology, RIKEN Research Center for Allergy and Immunology

Dendritic cells (DCs) are antigen-presenting cells characterized by a unique capacity to initiate primary immune responses and consist of

heterogeneous subsets. Recent studies suggest that DCs also play critical roles in the induction of peripheral immunological tolerance[1].

We examined the effects of immunostimulatory mature DCs(mDCs) and regulatory DCs (DC_{regs}) on T_H2-driven allergic immunity using a murine model of airway hyperresponsiveness. Treatment of antigen-primed mice with antigen-pulsed mDCs inhibited antigen-specific IgE production involving the generation of IL-21-producing T follicular helper(T_{FH}) cells but enhanced the production of antigen-specific IgG_1 and IgG_{2a}, and failed to suppress the airway's allergic response through the activation of diverse types of T_H cell responses. In contrast, antigen-pulsed DC_{regs} impaired the production of antigen-specific IgE, IgG_1 and IgG_{2a}, and antigen-specific $CD4^+CD25^+Foxp3^+$ regulatory T(T_{reg}) cells mainly mediated this suppressive effect. Antigen-pulsed DC_{regs} abolished T_H2-mediated allergic inflammation based on antigen-specific dominant tolerance[2].

We also examined the therapeutic utility of DC_{regs} in the major histocompatibility complex-compatible and multiple minor histocompatibility antigen-incompatible model of chronic graft-versus-host disease (cGVHD) in allogeneic bone marrow transplantation (alloBMT). Treatment of the recipient mice after alloBMT with the recipient-type DC_{regs} led to suppression of the incidence and severity of cutaneous cGVHD, whereas treatment with the recipient-type mDCs promoted the pathogenesis. The protective effect of the recipient-type DC_{regs} was mediated through the induction of a dominant tolerance involving the peripheral generation of alloreactive $CD4^+CD25^+Foxp3^+T_{reg}$ cells from donor-derived $CD4^+CD25^-Foxp3^-$T cells[3].

Thus, immunotherapy with DC_{regs} is a promising strategy for the treatment of allergic airway inflammation and cGVHD in alloBMT.

Publications

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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 30 patients per year receive allogeneic HSCT in our facilities. In recent years, unrelated cord blood has been our major stem cell source for recipients who have no suitable family donors in HSCT. Since 1998 we have performed over 150 cases of unrelated cord blood transplantation (uCBT) for adult patients, which appears a distinguished experience in the world. Recent advances in identification of signaling molecules activated in a tumor-specific manner or associated with tumor-specific genomic recombination have disclosed many candidate therapeutic targets in tumors. In the field of hematological malignancies, we have already experienced remarkable clinical efficacies of imatinib mesylate, an ABL kinase inhibitor, for CML as well as rituximab, a chimeric anti-CD20 monoclonal antibody for B cell lymphoma. We extensively apply these molecular targeted therapies for in- and out-patients and are to be involved in clinical trial of newly developed agents.

1. Impact of cytomegalovirus serostatus on outcome of unrelated cord blood transplantation for adults: a single-institute experience in Japan.

Tomonari A, Takahashi S, Ooi J, Tsukada N, Tojo A.

Cytomegalovirus (CMV) disease is one of the major infectious complications after allogeneic hematopoietic stem cell transplantation (SCT). Several studies have shown that CMV-seropositive patients have a substantial survival disadvantage after bone marrow transplantation (BMT) or peripheral blood SCT (PBSCT). Between August 1998 and February 2006, 101 adult patients underwent myeloablative cord blood transplantation (CBT) from unrelated donors at our institution. Sixteen and 85 patients were CMV-seronegative and CMV-seropositive,

respectively, prior to CBT. Outcomes of CBT were compared between CMV-seronegative and CMV-seropositive patients. The cumulative incidences of neutrophil engraftment at 60 d after CBT did not differ between CMV-seronegative and CMV-seropositive patients (100% and 94%, P=0.09); however, the cumulative incidence of platelet engraftment at 100 d was higher in CMV-seronegative patients than CMV-seropositive patients (100% vs. 86%, P<0.005). The cumulative incidence of CMV antigenemia at 100 d was lower in CMV-seronegative patients than CMV-seropositive patients (0% vs. 77%, P<0.001); however, the cumulative incidences of CMV disease did not differ between CMV-seronegative and CMV-seropositive patients (0% vs. 1%, P=0.84). The probabilities of disease-free survival at 2 yr also did not differ between CMV-seronegative and CMV-seropositive patients (92% vs. 72%, P=0.16). The outcomes of CBT for CMV-seropositive patients as well as CMV-seronegative patients in our series were favorable. This might be due to effective antiviral therapy for CMV infection. Large-scale studies are needed to determine the impact of recipient CMV serostatus on the outcome of CBT for adults.

2. Preemptive therapy with ganciclovir 5 mg/kg once daily for cytomegalovirus infection after unrelated cord blood transplantation.

Tomonari A, Takahashi S, Ooi J, Tsukada N, Tojo A.

The efficacy and safety of preemptive therapy using ganciclovir (GCV) 5 mg/kg once daily for CMV infection after unrelated cord blood transplantation (CBT) were studied. The initial preemptive therapy with GCV 5 mg/kg once daily led to resolution of CMV antigenemia in 25 of 34 patients (74%). In the remaining 9 patients (26%), antigenemia resolved after dose-escalation of GCV or change to foscarnet therapy. Recurrence of antigenemia was seen in 18 patients (53%). A total of 12 patients received the second preemptive therapy with GCV 5 mg /kg once daily, which led to resolution of antigenemia in 11 of 12 patients (92%). The remaining 1 patient (8%) required change to foscarnet therapy. None of 34 patients developed CMV disease. Neutropenia with an absolute neutrophil number of less than 1 and 0.5×10(9) per liter after GCV therapy occurred in 12 (35%) and 1 (3%) patients, respectively, after the initial therapy, and in 2 (17%) and 0 (0%) patients, respectively, after the second therapy. No patients developed neutropenic fever or secondary graft failure after GCV therapy. There were no deaths directly attributable to GCV therapy. The present study suggests that antigenemia-based preemptive strategy using GCV 5 mg/kg once daily is feasible and effective for CBT recipients.

3. Four cases of donor cell-derived AML following unrelated cord blood transplantation for adult patients: experiences of the Tokyo Cord Blood Bank.

Nagamura-Inoue T, Tojo A.

Donor cell leukemia (DCL) is considered as a rare complication following allogeneic bone marrow transplantation (BMT), whereas the actual frequency of DCL has not yet been specified. Cord blood (CB) is now recognized as an alternative source for stem cell transplantation (SCT), with more than 6,000 CBT performed worldwide, and a few cases of DCL following CBT have also been reported[1,2&3]. Here we report four cases of DCL developed after unrelated CBT using 478 units whose clinical reports were available among 596 units shipped from Tokyo cord blood bank (Tokyo CBB). Two cases out of the four have been already reported elsewhere[2&3] Development of DCL was informed to Tokyo CBB by attending physicians of the recipients in CBT centers soon after definite diagnosis was made. The feed-back from CBT centers on four DCL cases is summarized in Table 1. All the donors were well at the follow-up questionnaire of 6-12 months after birth, but further information of their health conditions has not yet been obtained. Notification of DLC to the donors' parents has not yet done because DLC in the recipient does not always mean occult leukemia in the donor and we do not desire to create the excess anxiety to the parents.

The etiology of DCL is unclear and a common mechanism is unlikely according to the reported literature[4,5&6]. There exist several possibilities including occult leukemia or preleukemic state in the donor, defect in immune surveillance, therapy-related stromal abnormalities, excess of cytokine stimulation, and DNA replication and/or repair errors associated with post-transplant expansion of stem/progenitor cells. In this regard, it should be noted that these four cases developed AML which is relatively rare in childhood acute leukemia[7], suggesting the extrinsic influences including excessive cytokine release, infectious agents and defected immunosurveillance on leukemogenesis in the CBT setting. Nevertheless, the possibility of occult leukemia in the donor raises serious problems regarding to the ethical responsibilities of the CBB to the donor. Ethical, but with scientific back-

Patient	1	2	3	4
Age/Sex	32/F	32/F	56/F	30/M
CB Sex	M	F	M	F
Diagnosis Status Biomarker	AML-M2 REL1 AML1-ETO (+)	AML-M0 REL1 (−)	ATL CR1 HTLV-1 (+)	Hodgkin's disease stage IVA (−)
Regimen	Myeloablative	Myeloablative	Reduced intensity	Reduced intensity
TBI	12Gy	12Gy	(−)	2Gy
G-CSF	(+)	(+)	(+)	(+)
GVHD prophylaxis	CsA+sMTX	CsA+sMTX	FK506+PSL	CsA+sMTX
aGVHD	II	II	0	III
cGVHD	(−)	(−)	(−)	Limited
DCL	AML	AML-M2	AML	AML-M5
Onset	15 M postCBT	11 M postCBT	7 M postCBT	16 M postCBT
Chimerism Diagnosis Blast Biomarker	Y-probe FISH 100% XY 84% in PB AML-1/ETO (−)	STR 100% donor type 13% in PB (−)	STR 100% donor type 93% in PB HTLV-1 (−)	STR 100% donor type 50% in PB MLL (+)

ground, discussion should be continued about the cause of DCL.

4. Impact of ABO incompatibility on engraftment and transfusion requirement after unrelated cord blood transplantation: a single institute experience in Japan.

Tomonari A, Takahashi S, Ooi J, Tsukada N, Tojo A.

The impact of ABO incompatibility between donor and recipient on engraftment and transfusion requirement was studied in 95 adults who underwent unrelated cord blood transplantation (CBT). The patients included 27 ABO-identical, 29 minor, 21 major and 18 bidirectional ABO-incompatible recipients. Neutrophil engraftment did not differ between ABO-identical/minor ABO-incompatible and major/bidirectional ABO -incompatible recipients (hazard ratio (HR) 1.17, $P=0.48$). Cumulative incidence of platelet engraftment in ABO-identical / minor ABO-incompatible recipients was higher than in major/bidirectional ABO-incompatible recipients (HR 1.88, $P=0.013$). In addition, fewer platelet transfusions were required during the first 60 days after CBT in ABO-identical/minor ABO-incompatible recipients (HR 0.80, $P=0.040$). RBC engraftment did not differ between the two groups (HR 1.25, $P=0.33$). However, fewer RBC transfusions were required in ABO-identical/minor ABO-incompatible recipients than in major/bidirectional ABO-incompatible recipients (HR 0.74, $P<0.005$). No patients developed pure red-cell aplasia after CBT. These results indicate that ABO incompatibility affected platelet engraftment and transfusion requirement of RBC and platelet in CBT recipients. Further studies including larger patient numbers are required to elucidate the impact of ABO incompatibility on the clinical outcome of CBT.

5. Bacterial bloodstream infection in neutropenic adult patients after myeloablative cord blood transplantation: experience of a single institution in Japan.

Tomonari A, Takahashi S, Ooi J, Tsukada N, Tojo A.

Bacterial infection is one of the most important causes of morbidity and mortality after unrelated cord blood transplantation (CBT). In the present study, we studied 101 adult patients with respect to the incidence, outcome, and risk factors for bacterial bloodstream infection (BSI) within 30 days after CBT using a myeloablative

conditioning regimen. Bacterial BSI occurred in 12 patients within 30 days after CBT. The cumulative incidence of bacterial BSI was 12%. The median time of onset was day +6 (range, day −1 to day +13) after CBT. In all patients, the neutrophil count was 0/microL at the onset of bacterial BSI. Eight (67%) and 4 (33%) of the isolates were Gram-positive and Gram-negative bacteria, respectively. Only 2 (17%) of the 12 patients who had bacterial BSI died within 100 days after CBT. No risk factors for the occurrence of bacterial BSI within 30 days after CBT were identified. The low mortality rate for bacterial BSI in the neutropenic period appeared to be associated with the low incidence (6%) of transplantation-related death at day +100 in our study patients. Early diagnosis of bacterial BSI and prompt treatment with effective antibiotics are necessary for neutropenic adult patients after myeloablative CBT.

6. Pancreatic hyperamylasemia and hyperlipasemia in association with cytomegalovirus infection following unrelated cord blood transplantation for acute myelogenous leukemia.

Tomonari A, Takahashi S, Ooi J, Tsukada N, Tojo A.

Cytomegalovirus (CMV)-associated pancreatitis is rare after allogeneic hematopoietic stem cell transplantation (SCT). We describe a patient who developed pancreatic hyperamylasemia and hyperlipasemia in association with CMV infection after cord blood transplantation (CBT). A 31-year-old man with acute myelogenous leukemia underwent CBT. A neutrophil count consistently greater than 500/microL was achieved on day +21. Positive results for CMV antigenemia on days +35 and +67 prompted 2 courses of preemptive therapy with ganciclovir or foscarnet. The CMV antigenemia value again became positive on day +134. On day +141, serum amylase and lipase activities markedly increased to 1221 IU/L and 894 IU/L, respectively. The patient had no abdominal symptoms. Ultrasonography and computed tomography results showed no abnormalities of the pancreas. A diagnosis of possible pancreatitis was made. After the initiation of foscarnet therapy, the CMV antigenemia results soon became negative, and serum amylase and lipase activities returned to normal. Therefore, CMV infection was considered to play a major role in the development of pancreatic hyperamylasemia and hyperlipasemia in our patient. The present report indicates that CMV infection should be included in the differential diagnosis for patients with pancreatic hyperamylasemia after SCT.

7. Posttransplantation engraftment and safety of cord blood transplantation with grafts containing relatively low cell doses in adults.

Takahashi S, Ooi J, Tomonari A, Tsukada N, Tojo A.

The cell dose of a graft is a critical determinant of hematopoietic recovery and survival following unrelated cord blood transplantation. Most studies have found that the minimum acceptable nucleated cell dose should be between 1.5×10^7 and 2.0×10^7 nucleated cells per kilogram of body weight to reduce the time to myeloid recovery and increase the probability of engraftment. For some patients who have indications for hematopoietic cell transplants and for whom no other graft source except cord blood is available, it is difficult to decide whether they can receive cord blood grafts containing lower cell doses. In our study, patients who received cord blood grafts containing 1.0×10^7 to 2.0×10^7 cells/kg (n=7) exhibited slower neutrophil and platelet recoveries compared with patients who received grafts containing total nucleated cell doses of 2.0×10^7 cells/kg and above (n=93); however, 4 of those low-cell-dose recipients survived with a longer follow-up. Based on these preliminary results, cord blood grafts containing less than 2.0×10^7 cells/kg may be useful for cases where no grafts with higher cell doses or other stem cell sources are available.

8. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen.

Takahashi S, Ooi J, Tomonari A, Tsukada N, Tojo A.

We studied the clinical outcomes of 171 adults with hematologic malignancies who received unrelated cord blood transplantation (CBT) as a primary unrelated stem-cell source (n=100), or bone marrow transplant (BMT) or peripheral blood stem-cell transplant (PBSCT) from related donors (n=71, 55 BMT and 16 PBSCT). All patients received myeloablative regimens including 12 Gy total body irradiation. We analyzed the hematologic recovery, and risks of graft-versus-host disease (GVHD), transplantation-related mortality (TRM) and relapse, and disease-free

survival (DFS) using Cox proportional hazards models. Significant delays in engraftment occurred after cord blood transplantation; however, overall engraftment rates were almost the same for both grafts. The cumulative incidences of grades III to IV acute and extensive-type chronic GVHDs among CBT recipients were significantly lower than those among BMT/PBSCT recipients. Multivariate analysis demonstrated no apparent differences in TRM (9% in CBT and 13% in BMT/PBSCT recipients), relapse (17% in CBT and 26% in BMT/PBSCT recipients), and DFS (70% in CBT and 60% in BMT/PBSCT recipients) between both groups. These data suggest that unrelated cord blood could be as safe and effective a stem-cell source as related bone marrow or mobilized peripheral blood for adult patients when it is used as a primary unrelated stem-cell source.

Publications

1 Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kato S, Kasahara S, Iseki T, Yamaguchi T, Tojo A, Asano S. Impact of cytomegalovirus serostatus on outcome of unrelated cord blood transplantation for adults: a single-institute experience in Japan. Eur J Haematol. 2007 Dec 19; [Epub ahead of print]

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Founded in 1981, Department of Infectious Diseases and Applied Immunology (DIDAI) started HIV clinic in 1986. In 2007, 40 new patients with HIV infection have visited or admitted our hospital and, in total, 352 patients are currently under our clinical management. The total number of in-patients during 2007 was 36, and 5-7 beds for HIV-infected patients and other disorders patients in infectious disease ward have been occupied. Since the number of the staff members of DIDAI is too small to care both outpatients and in-patients, members of the Division of Infectious Diseases and the Department of Infectious Disease Control join the clinic. 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.

Takashi Odawara, Takeshi, Fujii[1], Tokiomi Endoh , Tomohiko Koibuchi[1] , Hitomi Nakamura[1], Takuya Maeda[2], Yuichi Sakamoto, Mutsunori Iga[2], Takeshi Matsumura[1], and Aikichi Iwamoto[1,2]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious Disease Control, International Research Center for Infectious Diseases

a. Treatment of HIV infection in IMSUT hospital: Statistical characteristics of HIV infected patients in IMSUT hospital this year

Forty new patients with HIV-1 infection visited our hospital this year (from January 1 to December 31, 2007), and 352 patients in total are under medical management in our outpatient clinic. The total number of HIV-infected in-

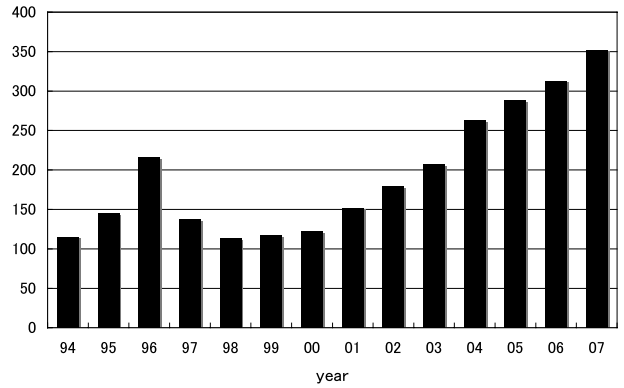

Figure 1. Number of HIV-infected patients in IMSUT Hospital

patients during 2007 was 36. The number of total patients declined in 1997 because a part of patients as well as medical stuffs moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again after 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 undetectable 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 control patients with HAART.

b. Clinical features of *Pneumocystis* pneumonia in patients with HIV infection

Pneumocystis pneumonia (PCP) remains the most common opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS). Familiarity with the clinical features of PCP is crucial for prompt diagnosis, even if the patient is unaware of their HIV serostatus.

This year, we reported the clinical features of 34 microbiologically confirmed episodes of PCP in 32 patients with HIV infection (2 patients developed PCP twice) who we treated in IMSUT hospital between 1995 and 2005 (1). In 20 of 32 patients (63%), HIV infection was diagnosed after the episode of PCP. As for symptoms, frequency of fever, cough and dyspnea was 71%, 74% and 65%, respectively, and the complete triad was only present in 14 of 34 episodes on first examination. Median duration from onset of symptoms until diagnosis was 3 weeks and AIDS-associated PCP tended to take an insidious clinical course. Although laboratory findings were generally nonspecific, measurement of β-D-glucan levels in the serum or plasma was highly useful in the diagnosis of PCP. All the patients except only 1 showed β-D-glucan levels higher than the cut-off value (median, 147 pg/ml; range, 5-6920 pg/ml). Serum lactate dehydrogenase (LDH) levels were commonly elevated (range, 153-1177 IU/l; median, 393 IU/l; mean, 473 ±259 IU/l): above the cut-off of 220 IU/l in 32 of 34 episodes (94.1%), however, elevated LDH should be explained with caution since it is also elevated in patients with various lung diseases, such as pulmonary tuberculosis and other bacterial pneumonias. Typical radiographic features for PCP are bilateral, symmetrical ground-glass opacities, but a wide variety of radiographic findings were observed. High-resolution computed tomography of the lung showed ground-glass opacities sparing the lung periphery (42%) or displaying a mosaic pattern (34%), nearly homogeneous ground-glass opacities (24%), consolidation (21%), cystic formation (21%), linear-reticular opacities (18%), patchy and irregularly shadowing (15%), solitary or multiple nodules (9%) and parenchymal cavity lesions (6%) in our cases.

c. Unusual manifestation of *Pneumocystis* pneumonia in an AIDS patient

Among a variety of pulmonary complications in acquired immune deficiency syndrome (AIDS), *Pneumocystis* pneumonia (PCP) is the most prevalent opportunistic infection. Typical radiographic features of PCP are bilateral perihilar interstitial infiltrates (ground-glass opacity) that become increasingly homogeneous and diffuse as the disease progresses. Although less common findings including focal infiltrations, nodules, cysts and cavitary lesions had been reported, the etiology and natural progress of these manifestations are still unclear.

This year, we reported an unusual PCP case in IMSUT hospital with AIDS who showed multiple nodular opacities with multilocular cavitations on chest computed tomography (CT) scan (2).

〈Case reports〉 A 52-year-old man was admitted to IMSUT hospital on February 2004 because of occasional hemoptysis. Two weeks before admission, the patient noted fatigue and cough with bloody sputum, and examination in a local hospital revealed abnormal chest radiograph and seropositivity for human immunodeficiency virus type-1 (HIV-1). On admission to our hospital temperature was 37.3 °C, pulse rate was 103 beats/min, and blood pressure was 124/90 mmHg. Physical examinations revealed oral candidiasis and bilateral cervical lymphadenopathy. Laboratory data showed a total white blood cell

(WBC) count of 2140/μl, C-reactive protein level of 0.66 mg/dl and β-D-glucan level of 56.5 pg/ml. Arterial blood gas analysis was normal on room air. RNA load of HIV-1 was over 760,000 copies/ml and CD4 positive cell count was 33/μl. Chest X-ray on admission showed a nodular lesion with cavitation at hilum of left lung and consolidations at middle right and lower left lung fields. A chest CT scan recorded 2 weeks before admission showed thick-walled cavitary nodules at left S6, right S6, right S10, and left S 10 with some focal infiltrations. A CT scan on admission revealed the cavitary nodules at left S 6 and right S10 which spontaneously changed into multiloculated cavities. Although Grocott staining of sputum was positive for *P. jiroveci*, we performed fiberoptic bronchoscopy to confirm that these atypical lung lesions were single infection with this microbe. Microscopic examination of bronchoalveolar lavage (BAL) fluid obtained from left B6 again showed *P. jiroveci* and no other microorganisms were detected by culture. Because we failed to get a sufficient tissue by transbronchial lung biopsy (TBLB), we carried out video-assisted thoracoscopic surgery (VATS) and excised a nodular lesion at left S10 for histopathological analysis. The excised lung tissue contained two distinctive areas, one of which was a solid parenchyma of alveoli filled with exudates and the other was an emphysematous area where alveolar septa were destroyed and alveoli were filled with air. Grocott staining of the exudates demonstrated numerous cysts of *P. jiroveci* that were positive for *P. jiroveci* in immunohistochemical staining. Notably, some bronchioles and its connecting alveolar ducts (AD) adjacent to emphysematous areas were surrounded by bulging alveoli that were filled with exudates, and upper stream bronchioles appeared distended. Treatment with trimethoprim / sulfamethoxazole (TMP / SMX) was started at a standard dosage. Chest CT about 3 weeks after initiation of treatment demonstrated that cavitary legions at left S6 and right S10 expanded with appearance of cystic formation and the cavity walls became thinner. All expanded cystic lesions had shrunken to tiny nodular legions and other nodular and infiltrative shadows had disappeared after a month of the completion of treatment, Although mechanisms of pulmonary cavitary lesions associated with PCP are still not clear, we speculate that check-valve phenomenon of small airways was responsible to generate cavities. It appears to be feasible that exudative parenchyma narrowed a part of small airways, some of which gave rise to check-valve and increased intraductal pressure in distal airways. In support of the speculation, histology of a left S10 lesion showed that cavitary areas were predominantly observed around exudative lesions with distended respiratory bronchioles and alveolar ducts.

d. Association analysis of *N*-acetyltransferase 2 gene polymorphism as a possible risk factor for adverse events of co-trimoxazole with HIV-positive patient.

Co-trimoxazole (trimethoprim- sulphamethoxazole) is an effective drug for prevention and treatment of *P. jirovecii* pneumonia that occurs in immunodeficient patients such as HIV infection. However, the usage of co-trimoxazole in HIV-positive patients is associated with very high frequency of adverse events including hypersensitivity and hepatotoxicity, which are reported in 40% to 80% of the patients. Of the two chemical components of co-trimoxazole, sulphamethoxazole is usually responsible for hypersensitivity. The major metabolic enzyme for sulphamethoxazole is *N*-acetyltransferase 2 (NAT-2). The absolute pathogenesis of the hypersensitivity is still unclear, but it is believed to involve the polymorphism of the *NAT-2* gene. We investigated the relationship of genetic polymorphism in the *NAT-2* gene and the incidence of adverse events in a Japanese HIV-positive people.

43 patients receiving primary prophylaxis had median CD4 count of 97.4 cells/ml and adverse events occurred in 13 patients (30.2%) with median CD4 count of 91.8 cells/ml. 15 patients (median CD4 count of 61.3 cells/μl) were administrated oral co-trimoxazole for the treatments of PC and only 4 patients (26.7%) of them (median CD4 count of 13.3 cells/μl) were free from adverse events. Adverse events occurred much fewer in those without the *NAT-2*^{*4} haplotype (1/7 [14.3%]) than in those with at least one *NAT-2*^{*4} haplotype (23/51 [45.1%]). Although all patients were evaluated with a clinical assessment and laboratory monitoring that including age, sex, steroids usage, CD4 count, HIV-RNA levels and genotyping of *NAT-2* gene, CD4 count was only associated with adverse event with co-trimoxazole in Japanese HIV-positive population.

A low CD4 count (<200/μl) with HIV infection is a risk factor for adverse event of co-trimoxazole, but the patients with excessive low CD4 count (<50/μl) become less susceptible to adverse event of co-trimoxazole. The allelic variations are unlikely to act as major determinants of adverse event of co-trimoxazole in a Japanese people with HIV.

2. Treatments and Clinical Research of Tropical Diseases

Takashi Odawara, Takeshi, Fujii[1], Tokiomi Endoh, Tomohiko Koibuchi[1], Hitomi Nakamura[1], Takuya Maeda[2], Yuichi Sakamoto, Mutsunori Iga[2], Takeshi Matsumura[1], and Aikichi Iwamoto[1,2]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious Disease Control, International Research Center for Infectious Diseases

a. Treatment of Tropical Diseases in IMSUT hospital

This year, 128 travellers visited our clinic for consultation or treatment of tropical diseases. Forty-four of 128 visited clinic before travel for prescription of malaria prophylaxis (24 travelers), vaccination (20 travelers; 17 for hepatitis A, 10 for hepatitis B, 13 for tetanus), and other general consultations (2 travelers). Eighty-four travelers visited clinic after travel because of sickness, and we diagnosed and treated 15 cases of traveler's diarrhea, 5 post-exposure prophylaxis of rabies, 4 malaria, 4 amoebiac enteritis, 3 dengue fever and 1 shigellosis.

b. *Fasciola hepatica* infection with huge cystic and multilocular lesions

F. hepatica is a trematode parasite that naturally infects cattle or sheep, and causes fascioliasis around the world. The infected young fluke, hatched from metacercaria, migrates in the peritoneal cavity and penetrates through the liver to the bile ducts causing the acute hepatic phase of fascioliasis. In the later stage, the fluke matures and lodges in the bile duct resulting in chronic biliary disorder. Although the radiological diagnosis of human fascioliasis has been improved, consideration of the possibility in the differential diagnosis is lacking in many developed countries. Typical computed tomography (CT) findings for hepatic phase of fascioliasis include small or clustered hypodense nodules and tortuous linear tracks, which are predominantly in subcapsular area.

This year, we reported a case with a unique hepatic phase fascioliasis. The patient was free from the symptoms, but presented uncommon radiological findings; a huge cystic lesion located in the middle of the liver together with peripheral multiloculated lesions (3).

A 61-year-old Japanese man was referred to the IMSUT hospital for the evaluation of migrating hepatic masses in November 2005. He had been involved in the construction of a power plant in Myanmar from January to November, 2004. A health checkup in January 2005 revealed blood eosinophilia and multiple hypo-echoic lesions in the right hepatic lobe by abdominal ultrasonography (US) imaging. A contrast-enhanced CT scan showed multiple hypodense lesions in the right hepatic lobe. In the anterior segment of the right lobe, a huge and low attenuated mass measuring up to 57 mm with regular margins and some tiny hypodense lesions were detected. Histological examination of the liver biopsy specimen was consistent with inflammation characterized by the presence of fibrotic changes. He was followed without any treatment and was referred to the IMSUT hospital. Contrast enhanced CT scans in November 2005, demonstrated mainly two types of masses in the right hepatic lobe. One of the masses, which had been detected in January but migrated during ten months, was located in the anterior segment and showed cyst-like hypodense lesion measuring up to 45 mm. The other mass, which had not been detected in January 2005, was located in the posterior segment and multiloculated. Magnetic resonance imaging (MRI) revealed hypointense lesions on T1-weighted images, hyperintense on T2-weighted images, and extremely hypointensive foci on inverted diffusion-weighted images. These MR images suggested that these hepatic lesions consistied of necrotic or abscess-forming materials. The diagnosis was made by serologic tests. We conducted a screening test for parasitic antibodies in the patient's serum using a multiple dot enzyme-linked immunosorbent assay (dot-ELISA). The antibody against *F. hepatica* was strongly positive by dot-ELISA. We also performed plate-ELISA and the ouchterlony double-diffusion test for confirmation. The ELISA titer for the antibody to *F. hepatica* was high and the ouchterlony test showed a strong precipitin band against the crude antigen of *F. hepatica*. The antibody to *Echinococcus multiocularis* was negative in plate-ELISA. The patient was treated with triclabendazole. After 6 weeks, abdominal CT revealed a significant decrease in the size of huge cystic lesion as well as the satellite lesions. The unique radiological findings mimicked hydatid diseases and also cystic liver neoplasms. Fascioliasis should be included in the differential diagnosis for cystic liver diseases.

c. Evaluation of the Rapid Diagnostic Tests for Malaria

Microscopic detection of malaria parasites on a blood smear is the gold standard for the diagnosis of malaria. However, it is still difficult for doctors with little experience. Rapid malaria di-

agnosing kits such as OptiMAL-IT™ (DiaMed, USA), NOW Malaria™ (Binax, USA), and pan-R MALARIA™ (Panbio, Australia) could be useful diagnostic tools.

This year we prospectively evaluated the effectiveness of pan-R MALARIA™ kit and 11 blood specimens derived from febrile returnees from malaria endemic areas. In all cases, microscopic examinations, pan-R MALARIA™ kit and OptiMAL-IT™ kit were performed. One of 11 cases ((9.1%) was microscopically confirmed as having falciparum malaria. Two rapid malaria diagnosis kits showed the same result. However, 3 of 11 cases (27.2%), who were microscopically negative for the malaria parasites, were *p. f*. positive by the pan-R MALARIA™ kit. They were simultaneously evaluated by OptiMAL-IT™ kit and PCR method for the detection of plasmodial DNA fragments. They were negative by either methods, and we excluded clinically. Hence, 3 false positive cases were experienced with pan-R MALARIA™ kit. Although the catalog of pan-R MALARIA™ kit shows that the sensitivity is 96.0% and the specificity of 99.7%, the sensitivity was 100% (1/1) and the specificity was 71.4% (8/11) in our study.

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Our major goal is to cure children suffering from a variety of life-threatening hematological disorders. Attempting to achieve it, 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 the diseases. Currently efforts are directed toward establishment of novel therapies including hematopoietic stem cell transplantation (HSCT) and regenerative medicine using human embryonic stem cells (hESC) and bome marrow (BM)-derived mesenchymal stem cells (MSC), and analysis of pathogenesis of hematopoietic disorders, especially pediatric myelodysplastic syndrome (MDS).

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

Yasuhiro Ebihara, Kohichiro Tsuji

Although a standard regimen in HSCT 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 (NK) 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 granulocyte colony-stimulating factor (G-CSF), and transplanted the patients whose leukemic cells had a high sensitivity to G-CSF using a regime including G-CSF. Thus, we could avoid intensive chemotherapy before HSCT for patients with a vulnerable normal bone marrow reserve. For patients with Fanconi anemia, in particular, 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 leukemia, 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-registant genes. Now we plan to extend our experience in nationwide collaborative studies.

2. Cooperative clinical trial for pediatric myelodysplastic syndrome

Kohichiro Tsuji, Yasuhiro Ebihara, Atsushi Manabe[1], Yuji Zaike[2]; [1]St. Luke's International Hospital, [2]Department of Laboratory Medicine, Research Hospital

Pediatric MDS is a rare disease, and only 50-100 children under the age of 16 suffer from the disease annually. The diagnosis and treatment

have not been standardized and it should be determined in a nationwide manner. On behalf of the MDS committee of the Japanese Society of Pediatric Hematology, we began the pathologic central review in 1999 and reviewed all samples of patients suspected of having MDS. At present, over 500 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 granulocyte-macrophage colony-stimulating factor (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. Novel approach to therapy in juvenile myelomonocytic leukemia

Yasuhiro Ebihara, Yoshitoshi Ohtsuka[3], Atsushi Manabe[1], Yuji Zaike[2], Kohichiro Tsuji; [3]Department of Pediatrics, Hyogo College of Medicine

JMML is a clonal myeloproliferative/myelodysplastic disorder of early childhood with poor prognosis. JMML cells are characterized by hypersensitivity to GM-CSF caused by continuously activated GM-CSF receptor-RAS signal transduction pathway through various molecular mechanisms, resulting in spontaneous colony formation *in vitro*. Bisphosphonate zoledronic acid (ZOL), a RAS-blocking compound, suppressed colony formation from BM cells of JMML patients and normal volunteers without and with GM-CSF, respectively, in a dose-dependent manner in clonal culture. At 10 mM of ZOL, however, spontaneous colony formation decreased, but formation of granulocyte (G) colonies containing only granulocytes, but no macrophages was enhanced in culture of JMML BM cells, while granulocyte-macrophage (GM) colonies containing both granulocytes and macrophages retained and G colony formation was not affected in culture of normal BM cells with GM-CSF. In suspension culture, 10 μM of ZOL also inhibited spontaneous proliferation and differentiation along monocyte/macrophage lineage of JMML BM cells, but not development of normal BM cells by GM-CSF assessed in cytochemical and flow cytometric analyses. The inhibitory effect of ZOL on JMML cells was confirmed at a single-clone level, and observed even at 3 μM. The current result offers a novel approach to therapy in JMML.

4. Development of B cell potential in the mouse embryo

Daisuke Sugiyama[4], Kohichiro Tsuji; [4]Department of Biochemistry, Dartmouth Medical School

Experiments using chimeric embryos in non-mammalian vertebrates demonstrated that mesodermally derived ventral compartments (yolk sac (YS) or its analog) and dorsal compartments (intraembryonic region) contribute to hematopoiesis in a different manner; primitive and definitive hematopoiesis, respectively. On the other hand, the ontogenic source of mammalian definitive hematopoiesis has remained controversial, because the *in utero* development of mammals excludes embryo grafting experiments. Results of earlier mouse studies led to the general acceptance of a model that murine definitive hematopoiesis begins in YS, shifts to fetal liver (FL), and finally resides in BM, in contrast to the conclusion derived from non-mammal vertebrates. However, recent studies have shown that early development of murine hematopoiesis is more complex than heretofore considered.

To investigate the ontogenic source of mammalian definitive hematopoiesis, we made mouse chimeras by grafting YS onto the YS of the host embryos before the establishment of circulation between YS and embryo proper, and cultured the whole embryo for 66 hours. Donor YS were taken from C57BL/6 Ly-5.1 and EGFP-transgenic mouse embryos, and recipient embryos from C57BL/6 Ly-5.2. Almost half of the grafts in YS-YS chimeras survived and had obvious blood flow, and the graft-derived cells achieved 12.7±0.9% of the blood cells in the circulation. These graft-derived blood cells consisted mainly of erythroid cells, some myeloid cells and a few blastic cells. Interestingly, CD19-positive B cells were generated from the graft-derived cells taken out from aorta-gonad-mesonephros (AGM) regions of the YS-YS chimeras but the frequency of the YS-derived B cells was low (1.0±0.6%) when co-cultured with OP9 stromal cells, demonstrating that B cell potential exists in YS before the circulation and that YS may also contribute to definitive lymphopoiesis *in vitro* in mice, while the major source for B cell in intra-embryonic AGM region.

5. Novel method for efficient production of multipotential hematopoetic progenitors from hESC

Feng Ma[5], Yasuhiro Ebihara, Sachiyo Hanada,

Yuji Zaike[2], Hiromitsu Nakauchi[5], Kohichiro Tsuji; [5]Laboratory of Stem Cell Therapy, Center for Experimental Medicine

ESC are pluripotent cells derived from the inner cell mass of preimplantation embryos. Since ESC have the ability to be maintained in culture indefinitely as undifferentiated cells, yet they are capable of forming more differentiated cell types, hESC recently established are expected as a novel source of human transplantable cells. We then planed to produce HSC for HSCT and functional blood cells for transfusion medicine from human ES cells. This study was started on December 20, 2003 with the permission by the ethical committee of the Japanese Government. On beginning this study, we thought that *in vitro* reconstitution of the circumstance surrounding embryonic hematopoietic cells is important to induce the differentiation of hESC into HSC or functional blood cells. To achieve this, we determined to use stromal cells from murine embryonic hematopoietic tissues to coculture hESC with them, since some mouse-derived stromal cells have been reported to be able to act on human hematopoietic cells.

We then proposed a novel method for the efficient production of hematopoietic progenitors from hESC by co-culture with stromal cells derived from murine FL (mFLSC) at 14 to 15 days post coitus (dpc), in which embryonic hematopoiesis dramatically expands at midgestation. In the co-culture, various hematopoietic progenitors were generated, and this hematopoietic activity was concentrated in cobblestone-like (CS) cells within differentiated hESC colonies. The CS cells expressed CD34 and retained a potential for endothelial cells. They also contained hematopoietic colony-forming cells , especially erythroid and multilineage colony-forming cells at high frequency. The multipotential hematopoietic progenitors abundant among the CS cells produced all types of mature blood cells, including adult type β globin-expressing erythrocytes and tryptase and chymase-double positive mast cells (MC). They showed neither immature properties of ESC nor potentials to differentiate into endoderm and ectoderm at a clonal level. The developed co-culture system of hESC can provide a novel source for hematopoietic and blood cells applicable to cellular therapies and drug screenings.

6. Generation of functional erythrocytes from hESC-derived definitive hematopoiesis

Feng Ma[5], Yasuhiro Ebihara, Sachiyo Hanada, Yuji Zaike[2], Hiromitsu Nakauchi[5], Kohichiro Tsuji

A critical issue for utilization of hESC in possible clinical use is whether they can derive terminally mature progenies with the normal function. To solve this, we examined hESC-derived erythroid cells in coculture with mFLSC. By the coculture, large quantity of hESC-derived erythroid progenitors allowed us to analyze the development of erythropoiesis at a clone level and to investigate their function as oxygen carrier. The results showed that the globin expression in the erythroid cells in individual clones changed in a time-dependent manner. In particular, embryonic ε globin positive erythrocytes decreased, while adult-type β globin positive cells increased to almost 100% in all single clones we examined, indicating they had already been fated to definitive hematopoiesis. Enucleated erythrocytes also appeared in the clonal erythroid progenies. A comparison analysis showed that hESC-derived erythroid cells took a similar pathway in differentiation to human cord blood $CD34^+$ progenitor-derived erythrocytes when traced by glycophorin A, CD 71 and CD81. Furthermore, these hESC-derived erythroid cells could function as oxygen carrier, and had a sufficient glucose-6-phosphate dehydrogenase activity. The present study provided an experimental model to explore early development of human erythropoiesis, hemoglobin switching, and erythroid pathogenesis, and to discover drugs for hereditary diseases in erythrocyte development. The quantitative production and their functional maturation indicate that hESC-derived erythrocytes can be a novel potential source for therapeutic transfusion.

7. Differential production of connective tissue-type and mucosal mast cells from hESC for anti-allergy drug screening

Feng Ma[5], Yasuhiro Ebihara, Sachiyo Hanada, Hiromitsu Nakauchi[5], Kohichiro Tsuji

MC function as effector cells in allergy and atopic disease. Therefore, anti-allergy drugs have been established to diminish MC function. However, since the acquisition of an abundance of human MC (hMC) is difficult because of no culture method producing massive hMC, most anti-allergy drugs targeted animal MC. Thus, efficient discovery of effective anti-allergy drugs needs to establish the culture system of massive hMC. Then, hESC are considered as a potential cell source for hMC.

In human, two types of MC have been characterized; connective tissue-type and mucosal MC (CTMC and MMC, respectively). CTMC contain tryptase, chymase, MC carboxypeptidase and cathepsin G in their secretory granules, are pre-

dominantly located in normal skin and intestinal submucosa, and involve in atopic dermatitis. MMC contain tryptase in their secretory granules, but lack the other proteases, are the main type of MC in normal alveolar wall and small intestinal mucosa, and involve in allergic rhinitis or bronchial asthma. Although MC can be generated from human adult $CD34^+$ hematopoietic progenitor cells *in vitro*, these MC are mainly MMC. So far, there lacks an evidence for the direct derivation of CTMC from adult hematopoietic progenitors.

We achieved successful production of hESC-derived $CD34^+$ hematopoietic progenitors, using co-culture with mFLSC for 1-2 weeks. In suspension culture favoring MC differentiation within 3weeks, hESC-derived progenitors generated mature MC that shared a chymase/tryptase double positive phenotype and strongly expressed c-Kit, similar to human skin derived CTMC. On the other hand, hESC-derived multipotential hematopoietic progenitors obtained in clonal culture developed into MC for a longer time (over 5 weeks) and only expressed tryptase, with no or few chymase, similar to human $CD34^+$ cell-derived MMC. Since the current culture system of hESC can produce differentially a large number of CTMC and MMC, our study may highlight a new understanding for MC development and finally benefit the screening for anti-allergy drugs.

8. Novel potentials of hematopoietic stem cells

Yasuhiro Ebihara, Makio Ogawa[6], Kohichiro Tsuji; [6]Department of Medicine, Medical University of South Carolina, USA

BM-derived stem cells have shown plasticity with a capacity to differentiate into a variety of specialized cells. To examine their novel potentials, we transplanted either isolated whole BM cells or clonally expanded HSC prepared from BM cells of enhanced green fluorescent protein (EGFP) mice into lethally irradiated congenic non-EGFP mice.

Quantification of $EGFP^+$ cells was performed 3-20 months after transplant. $EGFP^+$ cells were found in the inner ear with all transplant conditions. They were most abundant within the spiral ligament but were also found in other locations normally occupied by fibrocytes and mesenchymal cells. Dual immunofluorescence assays demonstrated that most of the $EGFP^+$ cells were negative for CD45, a macrophage and hematopoietic cell marker. A portion of the $EGFP^+$ cells in the spiral ligament expressed immunoreactive Na, K-ATPase, or the Na-K-Cl LO transporter (NKCC), proteins used as markers for specialized ion transport fibrocytes. In addition, histological analyses of valve tissue from clonally engrafted recipient mice revealed the presence of numerous $EGFP^+$ cells within host valves. A subpopulation of these cells exhibited synthetic properties characteristic of fibroblasts, as evidenced by their expression of mRNA for procollagen 11. These results demonstrate the HSC contribution to mesenchymal cells, including fibrocytes in the adult inner ear and valve.

9. Hematopoietic origin of fibroblasts (mesenchymal stem cells)

Yasuhiro Ebihara, Makio Ogawa[6], Kohichiro Tsuji

Using transplantation of a clonal population of cells derived from a single HSC of transgenic EGFP mice, we have documented the hematopoietic origin of myofibroblasts. Since myofibroblasts are thought to be an activated form of fibroblasts, we tested the hypothesis that fibroblasts are derived from HSC.

Clones of cells derived from single lineage-negative (Lin^-), c-kit^+, Sca-1^+, $CD34^-$ cells of EGFP Ly-5.2 C57Bl/6 mice were transplanted into lethally irradiated Ly-5.1 mice. Using BM and peripheral blood (PB) cells from mice showing high-level multilineage hematopoietic reconstitution, we induced growth of fibroblasts *in vitro*. Culture of $EGFP^+$ BM cells from clonally engrafted mice revealed adherent cells with the typical morphology of fibroblasts. Flow cytometric analysis revealed that the majority of these cells are $CD45^-$ and express collagen-I and the collagen receptor, discoidin domain receptor 2 (DDR2). RT-PCR analysis of the cultured cells demonstrated expression of procollagen 1-α1, DDR2, fibronectin and vimentin mRNA. Fibroblast colonies consisting of $EGFP^+$ cells were observed in cultures of BM cells from clonally engrafted mice indicating an HSC origin of fibroblast colony-forming units (CFU-F). Culture of PB nucleated cells from clonally engrafted mice revealed $EGFP^+$ cells expressing collagen-I and DDR2, indicating that fibrocytes are also derived from HSC. We then concluded from these results that a population of fibroblasts and their precursors are derived from HSC.

10. Establishment of human BM-derived MSC for the treatment of hemophilic arthropathy

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Hemophilia is the congenital disease with a lack of coagulation factors. One to two thirds of the patients had arthropathy because of recurrent intra-articular bleeding. Most of surgical treatment for the arthropathy, such as synovectomy or total joint arthroplasty, in Japan is performed by Department of Joint Surgery in our hospital. So far, however, the efficacy of the treatment has been insufficient. We then planed the transplantation of autologous culture-expanded BM-derived MSC into the articular cartilage defect in the hemophilic arthropathy patients. For the project, we are establishing the culture system of MSC from the patient BM using autolougous serum.

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We participate in cutting edge science of autoimmune, rheumatic and allergic disease and novel treatments for patients with these disorders. 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 and to perform the translational research.

I. Study on CD26 molecule in normal immune response and in patients with immune-mediated diseases

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CD26 is a T cell costimulatory molecule as well as an activation antigen with dipeptidyl peptidase IV (DPPIV) enzyme activity in its extracellular region that is preferentially expressed on memory T cells. The soluble form of CD26 (sCD26) is present in serum and recombinant soluble CD26 can enhance peripheral blood T cell proliferation induced by the recall antigen. We demonstrated that CD26 binds Caveolin-1 on antigen presenting cells, and that following CD26-caveolin-1 interaction on recall antigen-loaded monocytes, caveolin-1 is phosphorylated, with linkage to NF-κB activation, followed by upregulation of CD86. In addition, reduced caveolin-1 expression on monocytes inhibits CD 26-mediated CD86 upregulation and abrogates CD26 effect on recall antigen-induced T cell proliferation, and immunohistochemical studies revealed an infiltration of CD26+ T cells in the sublining region of rheumatoid synovium and high expression of caveolin-1 in the increased vasculature and synoviocytes of the rheumatoid synovium. Taken together, these results strongly suggest that CD26-caveolin-1 interaction plays a role in the upregulation of CD86 on recall antigen-loaded monocytes and subsequent engagement with CD28 on T cells, leading to antigen-specific T cell activation such as the T-cell-mediated antigen-specific response in rheumatoid arthritis (RA).

Recently we have developed the humanized anti-CD26 mAb for clinical application for

Immune-mediated disorders as well as CD26 positive Tumors. Hopefully we plan to perform phase I clinical trial against the above diseases this year.

a. Clinical significance of soluble CD26/DPPIV in various disease conditions

(i) Soluble CD26/DPPIV in autoimmune and other immune-mediated disorders

CD26 is a T cell costimulatory molecule as well as an activation antigen with dipeptidyl peptidase IV (DPPIV) enzyme activity in its extracellular region that is preferentially expressed on memory T cells. The soluble form of CD26 (sCD26) is present in serum and recombinant soluble CD26 can enhance peripheral blood T cell proliferation induced by the recall antigen. Our previous studies demonstrated that CD26-caveolin-1 interaction plays a role in the upregulation of CD86 on recall antigen-loaded monocytes and subsequent engagement with CD28 on T cells, leading to antigen-specific T cell. Possible substrates of CD26/DPPIV include several critical cytokines and chemokines. CD26 could modulate function of several cytokines and chemokines such as RANTES (CCL5), SDF-1α (CXCL12) and glucagons-like peptide 1(GLIP-1) through its DPPIV enzyme activity. We have shown that the DPPIV enzyme activity of plasma sCD26 was low in HIV-1-infected individuals, and was inversely correlated with HIV-1 RNA, and that the in vitro addition of recombinant sCD26 could enhance purified protein derivative-induced lymphocyte proliferation. These DPPIV enzyme activity of plasma sCD26 in HIV-1-infected individuals contributes to the immunopathogenesis of HIV infection. Furthermore, we have shown that serum levels of sCD 26 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. 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.

We examined sCD26 and its specific DPPIV activity in serum of patients with inflammatory bowel diseases (IBD), such as Crohn's disease or ulcerative colitis in collaboration with Gastrointestinal Unit, School of Medicine, Keio University. The DPPIV activity was reduced in patients with IBD and was significantly lower in patients with Crohn's disease compared to with ulcerative colitis ($P<0.05$). Serum levels of sCD26 and its specific DPPIV activity were significantly decreased in active disease of IBD ($P<0.001$). These findings indicate that CD26 may be potentially important for the pathophysiology of IBD, and appears to be useful as a new marker for disease activity in IBD.

We have also measured sCD26/DPPIV levels in sera and synovial fluid from patients with RA and found significant decrease of serum sCD26 and its specific DPPIV activity. We plan to examine the effect of TNF-α blocking therapy (infliximab or etanercept) on serum levels of sCD26 /DPPIV in patients with RA and its clinical significance.

(ii) Soluble CD26/DPPIV in malignancies associated with asbestos exposure

CD26 /DPPIV is able to cleave selected biological factors to alter their functions and regulates topoisomerase II α level in hematologic malignancies, affecting sensitivity to doxorubicin and etoposide. Expressed on various tissues, CD 26 is involved in the development of certain human cancers. We have shown CD26 is highly expressed on the cell surface of malignant mesothelioma and that a newly developed humanized anti-CD26 monoclonal antibody has an inhibitory effect on malignant mesothelioma cell gromth in both in vitro and in vivo experiments.

We examined sCD26 and its specific DPPIV activity in serum of patients with asbestosis in collaboration with Okayama Rosai Hospital. Serum levels of sCD26 and its specific DPPIV activity were significantly increased in patients with pleural plaque compared to healthy individuals ($P<0.05$). However, serum levels of sCD 26 and its specific DPPIV activity was significantly reduced in patients with both malignant mesothelioma and primary lung cancer associated with asbestos exposure compared to patients with pleural plaque. We are doing serial studies and measuring sCD26/DPPIV in pleural fluid to confirm their clinical significance.

b. Caveolin-1 triggers T-cell activation via CD 26 in association with CARMA1

CD26 is a widely distributed 110-kDa cell surface glycoprotein, having an important role in T-cell costimulation. We previously demonstrated that CD26 binds to caveolin-1 in antigen-presenting cells (APC), and that following exogenous CD26 stimulation, Tollip and IRAK-1 disengage from caveolin-1 in APC. IRAK-1 is then subsequently phosphorylated to upregulate CD86 expression, resulting in subsequent T cell proliferation. However, it is unclear whether caveolin-1 is a costimulatory ligand for CD26 in T-cell. Using soluble caveolin-1-Fc fusion protein, we now showed that caveolin-1 is the costimulatory ligand for CD26, and that ligation of CD26 by caveolin-1 induces T-cell prolifera-

tion and NF-κB activation in a TCR/CD3-dependent manner. We also demonstrated that the cytoplasmic tail of CD26 interacts with CARMA1 in T-cells, resulting in signaling events that lead to NF-κB activation. Ligation of CD26 by caveolin-1 recruits a complex consisting of CD26, CARMA1, Bcl10, and IKKβ to lipid rafts. Taken together, our findings hence provide novel insights into the regulation of T-cell costimulation via the CD26 molecule.

c. Humanized anti-CD26 monoclonal antibody as a treatment for malignant mesothelioma tumors

CD26 is a 110-kDa cell surface antigen with a role in tumor development. In this report, we show that CD26 is highly expressed on the cell surface of malignant mesothelioma and that a newly developed humanized anti-CD26 monoclonal antibody (mAb) has an inhibitory effect on malignant mesothelioma cells in both in vitro and in vivo experiments. Using immunohistochemistry, 12 patients' surgical specimens consisting of seven malignant mesothelioma, three reactive mesothelial cells, and two adenomatoid tumors were evaluated for expression of CD26. The effects of CD26 on malignant mesothelioma cells were assessed in the presence of transfection of CD26-expressing plasmid, humanized anti-CD26 mAb, or small interfering RNA against CD26. The in vivo growth inhibitory effect of humanized anti-CD26 mAb was assessed in human malignant mesothelioma cell mouse xenograft models. In surgical specimens, CD26 is highly expressed in malignant mesothelioma but not in benign mesothelial tissues. Depletion of CD26 by small interfering RNA results in the loss of adhesive property, suggesting that CD26 is a binding protein to the extracellular matrix. Moreover, our in vitro data indicate that humanized anti-CD26 mAb induces cell lysis of malignant mesothelioma cells via antibody-dependent cell-mediated cytotoxicity in addition to its direct anti-tumor effect via p27(kip1) accumulation. In vivo experiments with mouse xenograft models involving human malignant mesothelioma cells show that humanized anti-CD26 mAb treatment drastically inhibits tumor growth in tumor-bearing mice, resulting in enhanced survival. Our data strongly suggest that humanized anti-CD26 mAb treatment may have potential clinical use as a novel cancer therapeutic agent in CD26-positive malignant mesothelioma.

II. Therapeutically targetting transcription factors

Hirotoshi Tanaka, Noritada Yoshikawa, Noriaki Shimizu, Chikao Morimoto.

We are interested in the mechanism of eukaryotic gene expression and development of novel therapy and/or drugs 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 (GR) project

Glucocorticoid hormones are effective in controlling inflammation and immunity, but underlying mechanisms 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 in the anti-inflammatory process code for several cytokines, adhesion molecules. Most of them do not carry a classical binding site for regulation by the 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 genes for metabolic enzymes, expression of which have been shown to be positively regulated by the GR. We propose that a certain class of compounds (surprisingly, some of them are non-steridal 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 novel therapeutic strategy.

(i) Redox Regulation of the GR

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 glucocorticoid receptor belongs to a nuclear receptor superfamily and acts as a ligand-dependent transcription factor. We demonstrate that the glucocorticoid receptor 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 GR

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. Recently we have demonstrated that certain ligands can modulate interdomain communication of the GR, which will eventually contribute to isolation of novel category of ligands.

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. Our recent microarray study demonstrated that GR and MR have differential role in homeostatic regulation in non-classical corticosteroid target tissues including the heart. Notably, collaboration with Professor Miyano's laboratory greatly contributed to development of this program.

(iii) Molecular biology of small nuclear RNA binding protein HEXIM1

Expression of HEXIM1 is induced by treatment of vascular smooth muscle cells with a differentiation inducer hexamethylane bisacetamide. It is shown that HEXIM1 binds 7SK snRNA and inhibits P-TEFb-mediated transcriptional elongation process. On the other hand, we have found that HEXIM1 directly associates with the GR in the absence of 7SK and represses GR-mediated transcription. We are currently working on regulation of HEXIM1 expression, physiological role of HEXIM1 in GR action. Indeed, HEXIM1 has differential roles in gene regulation in a context and gene specific fashion. We have recently characterized that HEXIM1 may play an important role in tissue-specific regulation of glucocorticoid-mediated gene expression. Physiological significance of HEXIM1 is being studied using newly generated transgenic mice.

b. Hypoxia-inducible Factor (HIF)-1α 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.

Peripheral T cells encounter rapid decrease in oxygen tension as they are activated by antigen recognition and migrate into inflammatory sites or tumors. Activated T cells, therefore, are thought to have such machineries that enable them to adapt to hypoxic conditions and execute immune regulation in situ. We have recently shown that survival of CD3-engaged human peripheral blood T cells is prolonged under hypoxic conditions and HIF-1 and its target gene product adrenomedullin play a critical role for the process. It is also shown that hypoxia alone is not sufficient but TCR-mediated signal is required for accumulation of HIF-1α in human peripheral T cells. In the present study, we showed that TCR-engagement does not influence hypoxia-dependent stabilization but stimulates protein synthesis of HIF-1α, most possibly via PI3K/mTOR system, and that expression of HIF-1α and its target gene is blocked by treatment with rapamycin. Since some of those gene products, e.g., glucose transporters and phosphoglycerate kinase-1, are considered to be essential for glycolysis and energy production under hypoxic conditions and adequate immune reaction in T cells, this TCR-mediated synthesis of HIF-1α may play a pivotal role in peripheral immune response. Taken together, our results may highlight a novel aspect of downstream signal from antigen recognition by TCR with giving insight of a unique pharmacological role of rapamycin. We are currently working with the mechanism of translational regulation of HIF-1α.

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Our department was established to support the translational researches of our hospital. Currently, we are studying a correlation between expression profiles of peripheral blood mononuclear cells in patients with hematologic malignancy and severity of graft-versus-host disease developed after hematopoietic stem cell transplantation. We helped prospective studies of prediction of the sensitivity of Gefitinib to adenocarcinoma of the lung, and that of Imatinib to chronic myeloid leukemia (CML). In addition, we are in charge of outpatient clinic for genetic counseling in Research Hospital, IMSUT.

1. Analysis of gene expression profiles of inflammatory cells in acute graft-versus-host disease following umbilical cord blood transplantation

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Acute graft-versus-host disease (GVHD) is a common serious complication after allogeneic hematopoietic stem cell transplantation (HSCT), and is still a major cause of post-transplant mortality in patients with hematologic malignancy. In comparison with other sources of allogeneic HSCT, umbilical cord blood (UCB) transplantation (UCBT) offers substantial clinical advantages including both lower incidence and severity of acute GVHD despite the use of more HLA-disparate allogenic UCB stem cells. However, detailed pathophysiology of acute GVHD developed after UCBT remains unknown.

In order to identify the factors closely linked to the severity of acute GVHD following UCBT, we analyzed expression profiles of peripheral blood mononuclear cells using custom-made oligonucleotide arrays with a total of 615 genes that were selected from genes encoding cytokines, cytokine receptors, chemokines, chemokine receptors, adhesion molecules, and signal transducers. We compared expression profiles of four subsets ($CD4^+$, $CD8^+$, $CD14^+$, $CD56^+$) of peripheral blood mononuclear cells in acute GVHD with those counterparts in control phase. As a result, we identified several immunoregulatory genes that might link to the pathogenesis of acute GVHD.

2. Prediction of sensitivity of Gefitinib to lung cancer, and that of Imatinib to CML.

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In the second project, we are working on a prospective study of the prediction of sensitivity to Gefitinib (Iressa) in patients with lung adenocarcinoma, in collaboration with Human Genome Center, and Department of Applied Genomics in Institute of Medical Science Hospital. In our earlier study, we investigated expression profiles of lung adenocarcinomas that were treated with Gefitinib, which identified 12 genes that can discriminate tumors with sensitivity to the drug from those without sensitivity. Two other groups reported that genetic alteration of *EGFR*, the target of Gefitinib, was associated with the efficacy. Since these studies and ours analyzed a limited number of clinical samples, sensitivity and reliability of the two prediction methods remain unresolved. Therefore, we started a prospective study to analyze both expression profile and *EGFR* mutation of tumor tissues prior to treatment with Gefitinib, in collaboration with Human Genome Center, Applied Medicine, and Department of Infectious Diseases and Applied Immunology, in Research Hospital, IMSUT, and Kawasaki Medical University. An outpatient clinic for consultation of the applicants was opened in Research Hospital, IMSUT in September 2004, and department of respiratory medicine, Kawasaki Medical University joined to this project in 2005. In 2007, five patients were enrolled in this study after informed consent was obtained. Tumor specimens from the five were obtained by surgical operation, and all five samples were successfully analyzed.

In another study, we investigated expression profiles of 26 chromic myeloid leukemia (CML) with high-sensitivity and those with low-sensitivity to Imatinib (Glivec), and identified a total of 79 genes differently expressed between high- and low-sensitivity groups. We developed a prediction system of the sensitivity using expression of 15 genes among the 79. For the application of the findings into clinics, we launched a prospective study to evaluate the system, and analyzed expression profiles of additional CML samples in collaboration with Applied Medicine in Research Hospital, IMSUT. An outpatient clinic for consultation of applicants was opened in June, 2004, and one patient with CML visited the clinic in 2007. After written informed consent was obtained, blood samples were taken from the patient. FISH and expression profile analyses were carried out, and calculated prediction score of the sensitivity to Imatinib was reported to the patient in the clinic.

3. Genetic counseling and related activities.

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In the genetic counseling clinic, we provided genetic counseling for clients who suffered from or had family members of hereditary disease. Genetic diseases and related problems seen at the clinic in 2007 include Huntington disease, congenital deafness with cleft palate and severe myopia, hemophilia and genetic problems of stress tolerance. We also took psychological care of the clients in collaboration with clinical psychologists.

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The Department of Radiology works in general diagnostic radiology, neuroradiology, clinical nuclear medicine, radiation therapy, and molecular imaging. Image-based diagnosis and evaluation of therapeutic effect play essential roles in project-related treatments as well as in routine clinical practice. Molecular imaging techniques permit repeated assessment of individual animals to evaluate disease progression, therapeutic effect, and pharmacokinetics. They contribute to preclinical studies and have potentials of application to clinical assessments.

In vivo fluorescence imaging of the reticuloendothelial system using quantum dots: combination with bioluminescence tumor monitoring

Yusuke Inoue, Kiyoko Izawa[1], Kohki Yoshikawa[2], and Arinobu Tojo[1]: [1]Division of Molecular Therapy, Advanced Clinical Research Center, [2]Department of Radiotechnical Sciences, Faculty of Radiological Health Sciences, Komazawa University.

In vivo bioluminescence imaging (BLI) noninvasively visualizes the whole-body distribution and intensity of the luciferase gene expression using a sensitive CCD camera. After the inoculation of tumor model cells stably expressing luciferase, whole-body tumor burden can be assessed repeatedly and sensitively. However, determining involved organs is often difficult because of lack of anatomical information. On the other hand, the reticuloendothelial system can be visualized noninvasively by in vivo fluorescence imaging (FLI) using quantum dots (QDs). Many charge-coupled device (CCD) camera systems used for in vivo BLI can also be applied to acquire FLI images. Therefore, FLI may serve as a practical way to add anatomical information to BLI. We characterized in vivo FLI of the reticuloendothelial system using QDs and investigated its use in combination with in vivo BLI. In vivo FLI was performed in living mice repeatedly after the intravenous administration of QDs without conjugation to targeting ligands. Ex vivo FLI of the excised organs was performed 24 hours after QD injection in three mice. Seven days after intravenous inoculation of luciferase-expressing model cells of a hematological malignancy, mice were injected with the QDs or saline, and combined BLI/FLI was performed repeatedly. Other mice inoculated with the tumor cells were examined by in vivo BLI/FLI, and the structures harboring bioluminescent foci were determined by ex vivo BLI. The utility of combining FLI with bioluminescent tumor monitoring was evaluated. In vivo FLI after QD injection allowed long-term, repeated observation of the reticuloendothelial system in individual mice, although fluorescence intensity and image contrast gradually decreased over time. Ex vivo FLI verified selective accumulation in reticuloendothelial structures. The administration of QDs did not affect whole-body bioluminescent signal intensities during longitudinal tumor monitor-

ing. In vivo BLI/FLI, accompanied by fusion of both images, improved the accuracy and confidence level of the localization of the bioluminescent foci. In conclusion, in vivo FLI using QDs provides an overview of the reticuloendothelial system in living mice. In combination with bioluminescent tumor monitoring, fluorescent reticuloendothelial imaging is expected to provide valuable information for lesion localization.

Diet and abdominal autofluorescence detected by in vivo fluorescence imaging of living mice

Yusuke Inoue

Autofluorescence is a major problem in in vivo fluorescence imaging (FLI). In the far-red and near-infrared regions of the electromagnetic spectrum, the primary source of autofluorescence is known as chlorophyll and its metabolites in the intestine. We investigated the effect of diet on abdominal autofluorescence detected by in vivo FLI of living mice. Groups of mice were fed a regular, alfalfa-free, or purified diets, and whole-body FLI was performed without the administration of fluorescent probes. In addition, quantum dots were injected intravenously into mice fed one of the three diets, and FLI was performed 3 and 24 hours later. Intense autofluorescence originating from the animals' intestinal contents was observed in mice fed the regular diet. Intestinal autofluorescence declined prominently after feeding with the alfalfa-free diet and further after feeding with the purified diet. The decline was rapid and took only 1-2 days; however, it may have been affected by an intake of feces. The reticuloendothelial system was clearly delineated using a low dose of quantum dots in mice fed the purified diet, although intestinal autofluorescence was visible 24 hours postinjection in mice given the alfalfa-free diet and definitely impaired the image quality in mice fed the regular diet. The use of a low-fluorescent diet, especially a purified diet, rapidly reduces intestinal autofluorescence and is expected to enhance the potentials of in vivo FLI.

Improvement in imaging of living mice using a 1-T compact magnetic resonance imaging system

Yusuke Inoue, Kohki Yoshikawa[2], and Tomoyuki Haishi[3]: [3]MRTechnology Inc.

Although magnetic resonance imaging (MRI) is recognized as a powerful modality for small animal experiments, high cost and low research accessibility often preclude the use of MR imaging in biomedical experiments. A compact MRI system using a 1-T permanent magnet was introduced into the animal facility of the Institute. We improved hardware and software of the system, optimized imaging parameters and techniques, and, as a result, realized MRI of living mice with the system. Further improvement in image quality, convenience, and versatility are now being pursued. We introduced high-performance gradient coils to achieve shortening of echo time in TT1-weighted 3D FLASH imaging. After improving the capability of noise removal, minimum echo time was reduced from 3.6 ms to 2.2 ms. This allows favorable visualization of the liver even with high fat content and better demarcation of subcutaneous tumor with acceptable signal-to-noise ratio and small boundary effect. MRI is inherently affected by spatial distortion related to inhomogeneity in the magnetic field strength, and a system of correction for distortion is under development. The introduction of multislice spin echo T2-weighted imaging is planned. Our hope is that many researchers, especially those in the Institute, apply MRI to their studies and obtain fruitful results.

Development of a fusion imaging technique using bioluminescence imaging and magnetic resonance imaging

Yusuke Inoue, Yoshitaka Masutani[4], and Arinobu Tojo[1]: [4]Department of Radiology, Graduate School of Medicine, University of Tokyo.

In vivo bioluminescence imaging (BLI) detects luciferase expression in living mice easily and sensitively. However, because of projectional nature, lack of anatomical information, and low spatial resolution, the localization of bioluminescent sources is often difficult and unreliable. Magnetic resonance imaging (MRI) is another imaging technique applicable to small animal imaging and allows the assessment of detailed morphology. We have demonstrated the feasibility and usefulness of combined BLI and MRI evaluations in evaluating a mouse model of a hematological malignancy. For further sophistication of the multi-modality imaging approach, we are now studying to develop BLI/MRI fusion imaging. We made a mouse holder suitable for both BLI and MRI. MRI images have spatial distortion due to inhomogeneity in the magnetic field strength, and we made a three-dimensional grid phantom to estimate the distortion and software for rapid, three-dimensional conversion. A mouse model of localized hepatic tumor was established using a luciferase-expressing

human colon cancer cells, and preliminary testing on the model suggest the feasibility and utility of the BLI/MRI fusion imaging. We are pursuing establishment of a correction method for image distortion, development of a software package for registration and display, and validation of various models.

MRI assessment of lung parenchymal motion in normal mice and transgenic mice with sickle cell disease

Shigeru Kiryu, Tessa Sundaram[5], and Masaya Takahashi[6]: [5]Department of Bioengineering, University of Pennsylvania, [6]Department of Radiology, Beth Israel Deaconess Medical Center.

An in vivo MR-based biomechanical assessment in small animal scale may contribute to investigating the pathophysiology of pulmonary disorders and to evaluating therapy response. We investigated whether our MR-based method of parenchymal motion quantification was feasible to detect motional abnormalities associated with pathologic changes in small rodents. First, we attempted to establish the quantitative methodology necessary to evaluate regional lung motion in normal mice. Voxel-wise displacement vector field maps were generated between the end-inspiratory and end-expiratory coronal thoracic MR images on normal mice to analyze the magnitude and direction of parenchymal motion in the segmented regions. Normal mice revealed that the right and left lungs moved symmetrically but that there was greater movement in the lower than in the upper regions. Calculated strain was uniform in the entire lung. Second, transgenic mice with sickle cell disease were used to evaluate whether the method could quantitatively detect the effects of pulmonary sequestration due to short-term exposure to hypoxic conditions on lung motion. The analysis was repeated before and after short-term exposure to hypoxia to demonstrate the effect of hypoxia on the respiratory motion. In the transgenic mice, the pulmonary motion before hypoxia was similar to that observed in the normal. Upon exposure to hypoxia, the displacement magnitude reduced and the direction of motion in some areas became distorted. Thus, we proved that MR quantification of pulmonary motion was feasible in mice and the principle that the method could detect mechanical abnormalities due to pathologic changes. Quantification of pulmonary motion has potential to lead to earlier disease diagnosis and better monitoring of disease treatments.

The evaluation of hepatocyte specific contrast agents: Comparison of Gd-EOB-DTPA and Gd-BOPTA and the effect of the anesthesia

Shigeru Kiryu and Yusuke Inoue

MR imaging has become a novel method in the liver disease in research and clinical setting. Following the advent of the extracellular contrast agent, Gd-based hepatocyte specific contrast agents are introduced and has been evaluated. Among hepatocyte specific contrast agents, clinical trials are currently progressed with Gd-EOB-DTPA and Gd-BOPTA and the approval for clinical use was obtained in some nations. We previously reported the characteristics and utility of Gd-BOPTA for MR imaging of the mouse liver. As a next step, we evaluated Gd-EOB-DTPA for the application of mouse liver MR imaging in comparison with Gd-BOPTA. Mouse MR imaging was performed sequentially after the intravenous injection of Gd-EOB-DTPA and Gd-BOPTA under isoflurane anesthesia using a T1-weighted, three-dimensional fast low-angle shot (3D FLASH) sequence. To compare the effect of different anesthetic agent, sequential imaging after intravenous injection of Gd-EOB-DTPA under pentobarbital anesthesia was performed. Sequential imaging under the isoflurane and pentobarbital was also performed after subcutaneous injection of Gd-EOB-DTPA. The subcutaneous injection of Gd-EOB-DTPA was performed on awake mice. The appropriate dose of Gd-EOB-DTPA and Gd-BOPTA for subcutaneous injection was determined visually. The liver showed rapid enhancement after the intravenous injection of Gd-BOPTA and Gd-EOB-DTPA under anesthesia with isoflurane. Under anesthesia with pentobarbital, the enhancement of liver showed a similar pattern as that under isoflurane, however the steeper reduction after peak was observed. The liver showed gradual enhancement after subcutaneous injection of Gd-EOB-DTPA under anesthesia with isoflurane or pentobarbital; however, the peak contrast ratio differed between anesthetics. In the time course of Gd-EOB-DTPA after subcutaneous injection, the liver showed gradual enhancement, followed by rapid reduction in awake mice in comparison with anesthetized mice, implying that the washout of contrast agent from liver was accelerated in awake mice. Visual evaluation indicated that a dose of 0.1 mmol/kg was appropriate for clear delineation of the entire liver margin for both of Gd-EOB-DTPA and Gd-BOPTA. We clarified the difference between Gd-EOB-DTPA and Gd-BOPTA in the mice MR imaging. We also showed the effect of the anesthe-

sia in the imaging of mouse liver MRI.

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Research Hospital

Department of Surgery
外科

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Assistant Professor	Akira Kanamoto, M.D., D.M.S.	助　教	医学博士	金　本　　彰
Assistant Professor	Masahisa Jinushi, M.D., Ph.D.	助　教	医学博士	地　主　将　久

We have been engaged in the surgical treatment of solid tumors and the immunotherapy of various malignancies. We have also been offering diagnostic services, including endoscopic examination on upper and lower intestines and ultrasonic examination for hepato-biliary systems and the breast. One of the goals of our department is to provide evidence-based standard therapies including surgery, chemo-therapy, and radiation for cancer patients. However, more emphasis has been put on the development of the novel immunological and gene therapies in intimate collaboration with the Division of Bioengineering, Advanced Clinical Research Center. We have been conducting multiple early stage clinical trials (Phase I and II) for cancer patients at Research Hospital utilizing its fundamental functions enabling clinical research of high quality.

1. Summary of surgical treatment and other procedures in 2007

As shown in Table 1, major operations were performed in 50 patients with malignant diseases and with benign diseases.

Table 1. 50 major operations performed in 2006

GIST	1
Stomach	4
Colo-rectum*	8 (Colon: 6, Rectum: 2)
Small Intestine	4
Liver	2 (2metastatic liver cancer)
Biliary Tract	4
Pancreas	1
Thymoma	1
Breast	9
Thyroid	2
Ovary	2
Miscellaneous	15
Total	50

We especially endeavor in the treatment of the far advanced malignant tumors. In this period, the chemotherapy with imatinib against multiple liver metastasis of GIST, the resection of the peritoneal seeding and retroperitoneal metastasis around the kidney of GIST (genotyping showed the c-kit was wild type), chemoradio therapy against esophageal cancer with multiple lymph node metastasis and invasion to the bronchus, removing the ovarian metastasis of the gastric cancer combined chemotherapy and Caudate lobectomy against repeating metastatic liver cancer were performed.

2) Early Phase clinical trials of peptide vaccination against various types of cancer.

In intimate collaboration with the Division of Bioengineering, a number of early phase clinical trials have been initiated using epitope peptides identified by them and others. These epitope peptides include different types of antigens as described below.

a. New tumor-associated antigens (TAAs), which can induce potent cytotoxic T-cells (CTLs) specific to tumor cells, identified with genome-wide exploration using cDNA Microarray Profiling and reverse immunology strategy

b. Epitope peptides that were able to induce cellular immune responses against vascular endothelial growth factor receptor2 (VEGFR2), which has been shown to be one of the key factors in tumor angiogenesis.

c. Epitope peptides derived from gp100, a melanoma associated antigen

More than 15 of phase I or I/II clinical trials using the epitope peptides described above have been initiated to evaluate safety, immunological response and clinical response against advanced esophageal, gastric, colorectal, breast, and pancreatic cancer patients with HLA-A*0201 or HLA-A*2402. Most of the protocols were initiated after 2004, and more than 50 patients have been enrolled in these protocols as of 2007. All of the peptides were used with IFA in order to augment anti-tumor immunity. Candidates for entry into the trials have been properly selected in accordance with IRB-approved protocols, and the clinical outcomes including adverse events and anti-tumor effects were closely monitored by an independent council consisting of the third party members. The immunological response in these early phase trials have been carefully monitored in all patients to secure proof of concept (POC) of this type of vaccination protocol. These analyses have been performed by the Division of Bioengineering using multiple strategies including HLA multimer assay and ELISPOT assay for antigen-specific INF-γ production.

Some of these protocols have been completed and their results will soon be published. These results including very promising immune responses against the antigen peptides we used in the protocols.

3) Phase I clinical trials using various types of dendritic cells (DCs) to induce Th1 type immune responses

Dendritic cells (DCs) are very potent antigen-presenting cells, which play central roles in bridging between innate and acquired immunity via direct cell-cell interactions and/or cytokine production. Since these DC functions could be used to induce potent immune responses against specific antigens, clinical application of DCs has been initiated as a cellular immunotherapy against cancer. However, it has been reported that quality of the DCs used in the trial would critically affect the immune responses induced by the protocol. Thus, we have been testing fully matured DCs capable of T helper type 1 (Th1) polarization for which technologies have been developed in the Division of Bioengineering. The goals in this clinical trial using our propagated DC are to examine safety, immune responses, and further, the clinical efficacy associated with the peptide loaded fully matured DC vaccinations. The trial has been successfully initiated in close collaboration with the Core Facility for Therapeutic Vectors (CFTV). Immunological monitoring has been performed by the Division of Bioengineering

4) Gene therapy using DCs transfected with IL-12 genes

We are also in the process of initiating cancer gene therapy with intra-tumoral administration of dendritic cells transfected to secrete IL-12. Since a clinical grade adenoviral vector carrying IL-12 genes was produced by CFTV in 2007, we are planning to initiate the process to formally test the effects of this strategy in Phase I clinical trials.

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Research Hospital

Surgical Center
手術部

Associate Professor	Mieko Chinzei, M.D., M.D.Sc. (April 2007～) (Associate Professor Masakazu Hayashida, M.D., M.D.Sc.; October 2002～March 2007)	准教授	医学博士	鎮　西　美栄子 (2007年4月着任) (2002年1月—2007年3月：助教授　医学博士　林田眞和)
Assistant Professor	Sachiko Imamura, M.D.	助　教	医学士	今　村　佐知子
Clinical Engineer	Osamu Ichimura		臨床工学技士	市　村　　理

Our clinical practice and clinical as well as experimental studies have been focused on (1) anesthetic management in patients undergoing major surgery, (2) management of intraoperative and postoperative pain, and (3) management of chronic intractable pain, and (4) assessment of the impact of anesthesia and surgery on autonomic nervous activity. We have published several works on these subjects.

1. Safety in anesthetic management, especially focusing on cerebral circulation during anesthesia and surgery.

The Bispectral Index (BIS) is a recently developed derivative of processed electroencephalogram that has been proven to closely correlate with the level of consciousness during 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 also found that BIS is useful to detect cerebral ischemia during pediatric and adult cardiac surgery sepecially when used in combination with the near-infrared spectroscopy (NIRS) to measure oxygen saturation of the brain. Simultaneous monitoring with BIS and NIRS revealed that in children, especially in infants, cerebral ischemia occurred frequently during cardiac surgery presumably due to immaturity of the cerebral vascular autoregulation. We also reported successful anesthetic management of patients with compromised circulation.

2. Management of intraoperative and postoperative pain.

We have published several works on management of intraoperative and postoperative pain. 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/analgsics. We also published several review articles on how to manage postoperative pain, and original articles comparing various modalities of postoperative pain management.

3. Management of chronic intractable pain

We published several works on new treatment modalities for chronic intractable pain syndrome with various drugs including ketamine and ATP, after application of drug tests to differentiate the mechanism underlying the pain.

We also reviewed usefulness of epiduloscopy in pain management in patients with chronic intractable low back pain.

4. Assessment of the impact of anesthesia and surgery on autonomic nervous activity.

It is generally accepted that the parameters derived from power spectral analysis (PSA) of heart rate variability (HRV) can provide a non invasive measure of autonomic nervous activity. We published several works on assessment of the impact of anesthetics on autonomic nervous activity during perioperative period using real time monitor for PSA of HRV.

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Research Hospital

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

Project Lecturer Fumitaka Nagamura, M.D., D.M.Sc.
Project Assistant Professor Seiichiro Kobayashi, M.D., D.M.Sc.

研究拠点形成特任講師 医学博士 長 村 文 孝
研究拠点形成特任助教 医学博士 小 林 誠一郎

Division of Clinical Trial Safety Management (DCTSM) was established in 2001. The missions of DCTSM are risk management of the Research Hospital (RH) and the support and the monitoring of clinical studies conducting at RH, especially for Translational Research (TR). For protecting rights of participants to TR and conducting TR scientifically and ethically appropriate, we organized Translational Research Coordinator (TRC).

1. Risk management of Research Hospital

Fumitaka Nagamura, Seiichiro Kobayashi, Hatsue Narita

We engage in the risk management of RH. We have promoted the report system on medical incidents and accidents, and quick corresponding scheme such as "Medical Accident-Response Meeting" and "Council of Risk Management in the RH". We take place at least two seminars for staffs of RH on medical safety every year. Almost all of the worker at RH participated into these seminars. We issue annual manual on risk management and Standard Operating Procedures (SOP) on operations of RH. As the result of these actions, no severe medical accident has been observed since the foundation of DCTSM.

2. Assistance and oversight of TR

Fumitaka Nagamura

We assist and oversee the conduct of TRs at RH. The aim of them is to perform TRs ethically and scientifically appropriate. We discuss and advise on the protocols and written consent forms of TRs with principal investigators before submitting them to the Institutional Review Board. We return the repots within two weeks from the receipt, and the format of the reports is based on the manner of that in the U.S. FDA. Opinions are summarized into three sections: safety issue (most concern); major problem; and minor problems/suggestions. TRCs, which are consisted of research nurse, pharmacist, clinical psychologist, dietitian, and clinical laboratory technologist, also give their opinions on written consent forms. Major activities of TRC are: pre-explanation on TR before that from principal investigator; grasping the mental conditions and understandings of participants; conducting weekly TRC meeting which is discussed mainly on the status of participants. Although we have provided SOPs on every aspect for conducting TRs at RH, we are revising them so as to meet the current regulations and to make them easy-to -use.

3. Education on clinical studies for workers at Research Hospital

Fumitaka Nagamura

The major missions 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 problem in Japan. To educate workers of the hospital, as well as coordinators, the division took place the educational course on clinical studies. This meeting consists of 10 sections, and participants were required to resister for preparing the course materials. The content of this course consists of the basic knowledge of clinical studies, regulations, laws, coordinating skills, environment of clinical studies, and the system of the Research Hospital in terms of the conduction of Translational Research.

4. Effect of Conflict of Interest (COI) problems on the decision making of participants for translational research

Fumitaka Nagamura

Nowadays, the problem of COI has become one of the major concerns in the field of drug development and activities of academia. IMS has settled the leading-edge guideline. The point of IMS guideline is that principal investigators (PI) who have some factors on COI, such as an action as a board member of the related companies and the ownership of corporate stock of the related companies, will be allowed to act as PIs, if they obey the regulations which stated on the IMS guideline. These regulations are consisted of monitoring system on clinical trials and self-report system. This scheme has been developed so as to protect the right and safety of participants, however, how to disclose the information is still the question to be resolved. Adding to this problem, the information which may influence on the decision making of participants, especially for Translational Research, is also the question. We have been studying on these problems mainly by the hearing to participants. So far, almost all of the participants did not have concern on the COI problems, and showed the confidence the monitoring scheme of IMS.

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Research Hospital

Department of Cell Processing and Transfusion
セルプロセッシング・輸血部

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教 授 医学博士 東 條 有 伸
講 師 医学博士 長村(井上)登紀子

Our department is established in 1990, in order to manage the transfusion medicine and the cell processing for hematopoietic stem cell transplantation. Since 2004, we also joined the cell processing and banking as Tokyo Cord Blood Bank, Facility of Cell Processing and Cryopreservation, together with Division of Cell processing.
We have been engaged to study for the development of various cell therapies together with other departments, as follows.

1. Expansion and regulatory T cells and immune analysis:

Tokiko Nagamura-Inoue, Kazuo Ogami, Shin Nakayama[1], Kazuaki Yokoyama[1], Arinobu Tojo:[1]Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo

Regulatory T cells harbored the immunosuppressive effects and were related to the onset of graft-versus-host disease (GVHD), rejection of organ transplantation and autoimmune disease. We developed the system of *ex vivo* expansion of CD25＋FOXP3＋regulatory T cells from the small amount of peripheral blood, to apply the cell therapy for severe GVHD, autoimmune diseases (Collaboration with Division of Molecular Therapy).

2. Expansion of effector T cells against Ph1-positive leukemia:

Harntrasopwat Ratanakanit[1] , Tokiko Nagamura-Inoue, Kazuo Ogami, Arinobu Tojo:[1]Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo

This project is to aim to prevent the relapse of Ph1-positive leukemia using immunological cell therapy. We analyzed the immunological findings in the patients with Ph1＋CML treated with tyrosine kinase inhibitor and have been studied the expansion of antigen specific effector T cells using the unique antigen and culture system (Collaboration with Division of Molecular Therapy).

3. Analysis of the factor influencing on the cord blood transplantation:

Tokiko Nagamura-Inoue

In cord blood transplantation, there have been several problems including graft failure, relapse, GVHD, donor-derived leukemia. We analyze the factors influencing on these problems from the standpoint of cord blood banking.

4. Exploring mesenchymal stem cells derived from umbilical cord:

Ikuo Ishige[1], Tokiko Nagamura-Inoue, Arinobu Tojo: [1]Department of Stem cell processing, Lab. of Stem cell Therapy, The Institute of Medical Science, The University of Tokyo

In addition to contribute the research use of cord blood banking as the regenerative leading project, we have been explored the new source, mesenchymal stem cells derived from umbilical cord (Warton Jelly) (Collaboration with Division of Molecular Therapy and Department of Stem cell processing).

5. Room for Clinical Cellular Technology: RCCT:

Tokiko Nagamura-Inoue, Kazuo Ogami, Masako Hirai, Tsuneo A. Takahashi[1], Arinobu Tojo and RCCT Execution Committee: [1]Division of Cell Processing, The Institute of Medical Science, The University of Tokyo.

To promote the cell therapy related to translational research, RCCT has been established in 1997. Until now, following projects have been implemented; 1) Cord blood cell processing for banking (Tokyo Cord Blood Bank), 2) Dendritic cell therapy, 3)Regenerative therapy of alveolar bone derived from bone marrow mesenchymal cells, 4) Gene therapy for renal cancer.

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Research Hospital

Core Facility for Therapeutic Vectors
治療ベクター開発室

Professor	Hideaki Tahara, M.D., D.M.Sc.		教授(室長)	医学博士	田 原 秀 晃(併)
Project Associate Professor	Katsunori Sasaki, Ph.D.		特任准教授	医学博士	佐々木 勝 則
Project Assistant Professor	Hisako Katano, D.D.S., Ph.D.		特任助教	歯学博士	片 野 尚 子

The primary function of the Core Facility for Therapeutic Vectors (CFTV) is to support clinical trials that require the genetic modification and/or ex vivo manipulation of patients' tissue or cells under current Good Manufacturing Practice (cGMP) conditions defined by FDA of USA. The CFTV is the first facility established in Japanese academia to produce genetic or cellular vectors of clinical grade. Using this facility, clinical lots of the adenoviral vector and herpes vector are scheduled to be produced in CFTV in 2008.

1. Preparation of Standard Operating Procedures (SOPs)

The cGMP compliance is maintained using written SOPs prepared by ourselves. The SOPs codify all aspects of laboratory activities including facility design and operations of the personnel. The SOPs enables the staff not only to produce the reagents with high quality in the stable manners but also to help identify areas for improvement.

2. Adoption of ISO

In order to continuously improve our activities, quality management system of the CFTV has been assessed and found to be in accordance with the requirements of the quality standards detailed ISO9001:2000; in the scope of development and manufacture of cell and gene therapy products.

3. Validation of CFTV

The CFTV consists of two distinct units; 1) Vector Unit, the primary viral vector production suite which may also function as *ex vivo* transduction suite; 2) Cell Unit, cell processing suite capable of generating dendritic cells for immunotherapy and gene therapy. There are two self-contained vector production suites in the Vector Unit and two self-contained tissue culture suites in the Cell Unit. These suites are kept Class 10,000. There are many features incorporated into the design of this CFTV to minimize the risk of cross-contamination between products; i. e., unidirectional traffic flow, individual airlocks to each production suite, single-pass HEPA filtered supply air, 100 percent exhaust from the biological safety cabinets through dedicate ducts, among others. Periodical validation has been performed on the facility and the equipments in CFTV to ensure cGMP compliance.

4. Projects in CFTV

Four projects are now in progress in the CFTV.

I. Cancer gene therapy using dendritic cells transfected with IL-12 genes

Collaboration with Division of Bioengineering of Advanced Clinical Research Center (ACRC)

and Department of Surgery of the research hospital.

· **Preparation of the First Clinical Lot**

We have been preparing the replication-defective recombinant adenoviral vector encoding human interleukin-12, which is an immunestimulatory cytokine. The backbone of this vector is based on the E1- and E3-deleted serotype 5 adenovirus with a modified fiber, harboring an integrin-binding CDCRGDCDC-motif within the HI-loop of its knob protein. The IL-12 genes are driven by a CA promoter (CMV-IE enhancer with the chicken β-actin promoter). The master virus seed stock (MVSS) and purified final vector reagent have been successfully prepared and are now in the process of quality confirmation for use of early phase trials.

II. Vaccine therapy with peptide-loaded dendritic cells for advanced melanoma

Collaboration with Division of Bioengineering of ACRC and Department of Surgery of the research hospital.

· **Preparation of Peptide-Loaded Dendritic Cells (DCs)**

We have been supporting phase I clinical trials against melanoma. Based on the results of the basic research performed in Division of Bioengineering, the SOPs of the DC preparation have been written and used. The cellular reagents have been successfully prepared in the Cell Unit and offered for clinical trials without serious problems.

III. Oncolytic viral therapy using genetically engineered herpes simplex viruses for malignant brain tumors.

Department of Neurosurgery, Graduate School of Medicine, The University of Tokyo.

· **Manufacture of the viral vector**

In collaboration, we have been preparing oncolytic herpes simplex virus. This preparation will be used for phase I clinical trial for brain cancer patients. We have supported the establishment of the master and working cell banks of Vero cells to produce genetically engineered herpes simplex viruses. The cGMP compliant MVSS, which contains a replication-competent herpes simplex virus type 1 vector defective for the α47 gene, was successfully produced. The purified final products are soon to be generated.

IV. Development of robotized cell culture system

In collaboration with Dr. Wakitani of Osaka City University and Kawasaki Heavy Industries, Inc., we are developing robotized cell culture system which could be applied to a variety of procedures including virus production as a funded project by NEDO.

5. Financal Support

This CFTV has been supported in large by 21 st Century COE Program (P.I. Dr. Yusuke Nakamura) from Japan Society for the Promotion of Science (2003-2007). From 2007, it has been also supported by Coordination, Support and Training Program for Translational Research from Ministry of Education, Culture, Sports, Science and Technology (2007-2011).

Research Hospital

Department of Laboratory Medicine
検査部

The Department of Laboratory Medicine consists of eight divisions-clinical physiology, hematology, biochemistry, serology, bacteriology, molecular diagnosis and pathology, and a division of flow cytometrical analysis which has been added recently. This Department engages in the laboratory analysis and gives diagnosis of clinical materials in the hospital. While facilitating the ongoing translational research projects in the research hospital, the Department functions as an integrated diagnosis & monitoring laboratory that evaluates the safety and effectiveness of experimental therapeutic approaches.

Associate Professor Naoki Oyaizu, M.D, Ph.D
Assistant Professor Naouki Isoo, MD, Ph.D.

准教授，部長 医学博士 小柳津 直 樹
助 教 医学博士 磯 尾 直 之

Our basic research strategies include the following approaches: characterizing molecular mechanisms underlying the pathology, developing a novel method to measure the disease-defining mechanism in the clinical materials and evaluating the effectiveness of molecular-targeted therapies thereby contributing to the translational research conducted in the institute. Integrating molecular-/biochemical-based laboratory assays on the solid background of pathological examinations enables us to evaluate the effectiveness of experimental clinical trials and leads to correct experimental therapies that further promote translational research. Our department also functions as an integrated diagnosis & safety-monitoring laboratory as well as the division of quality control by examining/evaluating the safety of investigational new drugs under GMP conditions.

1. Pathological evaluation of cancer immunotherapy

We have initiated the analysis of surgical specimen obtained from the patients under cancer immuno-therapy conducted in the research hospital. By applying sophisticated immunohistochemical techniques, we now are 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. One of our goals is to evaluate the effectiveness of the therapies and to elucidate the mechanisms of anti-tumor immune response elicited by the therapy *in situ*.

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

We found the presence of characteristic pathological findings in bone marrow specimen from some patients with MDS-RA, aplastic anemia, or pure red cell aplasia, which implicates that common immunopathological mechanism, may be operative in these hematological abnormalities; that is destruction of erythroid precursors by immune-based mechanisms in the bone marrow. In collaboration with the Department of Hematology, the Department of Laboratory Medicine will elucidate molecular mechanisms based on

the pathological consideration to establish new disease entities and develop new therapeutic interventions.

3. Analysis of the chimeric gene expression of hematological disorder

We have initiated the analysis of bcr-abl gene expression in specimen from patients with CML and Ph1+ve ALL by real-time PCR and nested RT-PCR techniques. In addition, we sequenced the amplified products to provide information for the molecular resistance to STI571 treatment.

4. Developing quick & inclusive diagnosis system for infections disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions have drastically altered drawing attention. This has resulted in altered recognition and molecular interaction of infected cells with immune cells, leading to atypical pathological as well as clinical manifestations. While distinguishing infectious disease and immunological disorder calls for urgent attention, it may be difficult to achieve these tasks in some cases due to modified manifestations. To avoid such cases, it is imperative to establish a comprehensive diagnosis system of infectious disease to the earliest possible opportunity.

5. Immunopathogical analysis of hematopoietic cell transplantation

The number of allogeneic hematopoietic stem cell transplantation (HSCT), mainly cord blood transplantation, has been performed for the treatment of hematological malignancies. Graft-versus-host disease (GVHD), a life-threatening complication, occurs as a complication of allogeneic HSCT. Our prime goal is to develop a new way to detect GVHD and make an accurate evaluation of GVHD at our laboratory.

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