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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. Based on our achievement as a main hub of HSCT centers in Japan, we greatly contributed to establish the Japan Marrow Donor Program (JMDP) and have been continuously working for JMDP in not only transplantation but also collection of unrelated donor marrows. In recent years, unrelated cord blood has turned to be our major stem cell source in HSCT. Since 1998 we have performed up to 100 cases of unrelated CBT for adult patients, which appears a distinguished experience in the world.

1. Ganciclovir-related neutropenia after preemptive therapy for cytomegalovirus infection: comparison between cord blood and bone marrow transplantation.

Tomonari A, Takahashi S, Ooi J, Uchimaru K, Tojo A.

We studied ganciclovir (GCV)-related neutropenia after preemptive therapy for cytomegalovirus infection: 9 of 17 (53%) cord blood transplantation (CBT) patients and 18 of 20 (90%) bone marrow transplantation (BMT) patients developed GCV-related neutropenia with an absolute neutrophil count (ANC) of less than 1,000/μl. Among the patients who did not receive granulocyte colony-stimulating factor, 2 (13%) and 1 (7%) CBT patients, and 10 (56%) and 8 (44%) BMT patients, developed neutropenia with an ANC of less than 500 and 250/μl, respectively. The incidences of neutropenia in patients with an ANC of less than 1,000, 500, and 250/μl were significantly lower after CBT in comparison with BMT. Two BMT patients, but no CBT patients, developed neutropenic fever, and both patients recovered after antibiotic therapy. In CBT patients, a creatinine clearance rate of less than 50 ml/min and an absence of steroid therapy were associated with a greater incidence of GCV-related neutropenia. No risk factors for GCV-related neutropenia were found in BMT patients. These results suggest that GCV may be less toxic to myeloid progenitor cells from cord blood than those from bone marrow.

2. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult

patients with hematologic malignancies.

Takahashi S, Ooi J, Tomonari A, Uchimaru K, Tojo A.

Unrelated cord blood transplantation (CBT) has now become more common, but as yet there have been only a few reports on its outcome compared with bone marrow transplantation (BMT), especially for adults. We studied the clinical outcomes of 113 adult patients with hematologic malignancies who received unrelated BM transplants (n=45) or unrelated CB transplants (n=68). We analyzed the hematopoietic recovery, rates of graft-versus-host disease (GVHD), risks of transplantation-related mortality (TRM) and relapse, and disease-free survival (DFS) using Cox proportional hazards models. The time from donor search to transplantation was significantly shorter among CB transplant recipients (median, 2 months) than BM transplant recipients (median, 11 months; P<.01). Multivariate analysis demonstrated slow neutrophil (P<.01) and platelet (P<.01) recoveries in CBT patients compared with BMT patients. Despite rapid tapering of immunosuppressants after transplantation and infrequent use of steroids to treat severe acute GVHD, there were no GVHD-related deaths among CB transplant recipients compared with 10 deaths of 24 among BM transplant recipients. Unrelated CBT showed better TRM and DFS results compared with BMT (P=.02 and P<.01, respectively), despite the higher human leukocyte antigen mismatching rate and lower number of infused cells. These data strongly suggest that CBT could be safely and effectively used for adult patients with hematologic malignancies.

3. Unrelated cord blood transplantation after myeloablative conditioning in patients over the age of 45 years.

Ooi J, Takahashi S, Tomonari A, Soda Y, Ohno N, Uchimaru K, Tojo A.

We report the results of unrelated cord blood transplantation (CBT) after myeloablative conditioning in 21 patients over the age of 45 years. Among the patients the median age was 48 years (45-53), the median weight was 58.6 kg (range, 43.6-76.2 kg) and the median number of cryopreserved nucleated cells was 2.45×10^7/kg (range, $1.63\text{-}3.71\times10^7$/kg). Nineteen patients had myeloid reconstitution and the median time to more than 0.5×10^9/l absolute neutrophil count was 22 d. A self-sustained platelet count more than 50×10^9/l was achieved in 17 patients at a median time of 49 d. Acute graft-versus-host disease (GVHD) above grade II occurred in 7 of 19 evaluable patients and chronic GVHD occurred in 14 of 16 evaluable patients. Among 14 chronic GVHD patients, in seven patients the disease was extensive. Fifteen patients were alive and free of disease at between 217 and 1798 d after transplantation. With a median follow-up of 847 d, the probability of disease-free survival at 2 years was 71.4%. These results suggest that patients over 45 years of age without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.

4. Unrelated cord blood transplantation for adult patients with acute lymphoblastic leukemia.

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

13 adult patients with ALL were treated with unrelated CBT at IMSUT hospital. 10 patients received total body irradiation (TBI) and cyclophosphamide. Two patients received TBI, high dose cytosine arabinoside, and fludarabine. One patient received TBI, fludarabine, and melphalan. All patients received standard cyclosporine and methotrexate as a GVHD prophylaxis. Among the 13 patients, the median age was 36 y/o (18-53), the median weight was 56 kg (40-71), and the median number of infused nucleated cells was 2.89×10^7/kg (1.81-3.66). Six patients were beyond CR1 at transplantation. A total of 11 patients had myeloid reconstitution and median time to neutrophil recovery was 20 days (17-26). RBC recovery was achieved in 8 patients at a median time of 52 days (43-90). Platelet recovery was achieved in 10 patients at a median time of 48 days (40-106). All patients with myeloid reconstitution showed full donor chimerism. Acute GVHD occurred in all the evaluable patients. The grading of acute GVHD was grade I in five patients and grade II in six. Chronic GVHD occurred in 7 of 8 evaluable patients. Among 7 chronic GVHD patients, one patient was extensive type. Five patients died (one; hepatic VOD, four; relapse). Primary graft failure occurred in one patient. Seven patients are alive and free of disease between 133 and 1253 days after transplantation. With a median follow-up of 308 days, the probability of DFS was 55.9 %. At present, the role of unrelated cord blood as an alternative stem cell source is not well defined in adult ALL patients eligible for conventional conditioning regimens. The DFS rate in this study was similar to previously published reports of adult ALL patients who received HLA-matched sibling allogeneic transplantation. As the number of cohort was small, we could

not conclude that unrelated cord blood can act as an alternative stem cell source in adult patients with ALL. Nevertheless, these results suggest that adult ALL patients without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.

5. Unrelated cord blood transplantation with a reduced-intensity conditioning regimen following autologous transplantation for multiple myeloma.

Tomonari A, Takahashi S, Ooi J, Uchimaru K, Tojo A.

Two patients, 51- and 45-year-old men with stage III immunoglobulin G multiple myeloma, achieved partial and complete remissions, respectively, after conventional chemotherapy. They both received high-dose melphalan (200 mg/m^2) with autologous stem cell transplantation (ASCT). Eighty-four and 78 days after ASCT, the patients underwent unrelated cord blood transplantation (CBT) following treatment with total-body irradiation (2 Gy), fludarabine (90 mg/m^2), and melphalan (140 mg/m^2). Neutrophil engraftment was attained on day +27 in patient 1 and day +15 in patient 2. Full donor chimerism of the marrow cells was confirmed. Regimen-related toxicity in both patients remained within grade I. Grades I and II acute graft-versus-host disease (GVHD) occurred in patients I and 2, respectively, but improved without steroid therapy. Both patients developed limited chronic GVHD of the skin but needed no treatment. The serum paraprotein level in patient 1 decreased further after ASCT and CBT but remained at minimally detectable levels. The serum and urine paraprotein levels in patient 2 remained below detectable limits. These results suggested that CBT with a reduced-intensity conditioning regimen after high-dose chemotherapy with ASCT is a new promising approach for the treatment of multiple myeloma.

6. $CD34^+CD7^+$ leukemic progenitor cells may be involved in maintenance and clonal evolution of chronic myeloid leukemia.

Kosugi N, Tojo A.

We analyzed $CD34^+$ cells co-expressing CD7 in chronic myeloid leukemia (CML) in chronic or accelerated phase (CP or AP) to clarify their role in progression or regression of the disease during treatment. Enumeration of $CD34^+CD7^+$ cells was performed on bone marrow nucleated cells from normal donors and CML patients. Fluorescence *in situ* hybridization (FISH) analysis was performed on sorted $CD34^+CD7^+$ and $CD34^+CD7^-$ cells to examine the occupancy rate of each fraction by $BCR\text{-}ABL^+$ cells with or without additional cytogenetic abnormalities. The proportion of $CD34^+CD7^+$ cells was significantly affected by the treatment outcome and/or the disease status as follows: $20.5\pm10.4\%$ in normal donors ($n=22$); $18.1\pm10.2\%$ in CP with major cytogenetic response ($n=14$); $53.0\pm12.9\%$ in CP at diagnosis ($n=18$); $55.0\pm15.8\%$ in CP with no or minor cytogenetic response ($n=28$); $70.2\pm18.1\%$ in AP ($n=6$). The proportion of $CD34^+CD7^+$ cells decreased in parallel with cytogenetic improvement in individual patients. In 6 untreated CP patients, the ratio of $BCR\text{-}ABL^+$ cells was comparable between each fraction. In 3 patients with major cytogenetic response, the ratio of $BCR\text{-}ABL^+$ cells was remarkably lower in $CD34^+CD7^-$ cells than in $CD34^+CD7^+$ cells. In 3 AP patients with additional cytogenetic abnormalities, extra signals were detected at much higher rate in $CD34^+CD7^+$ cells than in $CD34^+CD7^-$ cells. Our results suggest that $CD34^+CD7^+$ cells may be involved in maintenance and clonal evolution of $BCR\text{-}ABL^+$ cells in CML.

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Department of Infectious Diseases and Applied Immunology (DIDAI) was founded in 1981. In 1986, clinic for patients with human immunodeficiency virus (HIV) infection was opened by former professor, K. Shimada. In 2004, 48 new patients with HIV infection have visited or admitted our hospital and, in total, 263 patients are currently under our clinical management. The total number of in-patients during 2004 was 45, and 5-7 beds for HIV-infected 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 (DID) and the Division of Clinical Immunology of the Advanced Clinical Research Center join the clinic. Supported by clinicians of three department and divisions, basic scientists of immunology and virology in DID, and dedicated medical and paramedical stuffs, IMSUT hospital provides the most up-to-date medical treatment to HIV-infected patients in Japan. DIDAI is also a treatment center for international infectious diseases such as malaria and typhoid fever.

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

Tetsuya Nakamura, Takashi Odawara[1], Takeshi Fujii[1], Tokiomi Endoh[1], Jun-ichi Takeda[1], Fuyuaki Ide[1], Takeshi Matsumura[1], Hitomi Nakamura[1], Miou Sato[1], Mieko Goto[1] and Aikichi Iwamoto[1]: [1]Department of Infectious Diseases

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

Forty-eight new patients with HIV-1 infection visited our hospital this year, and as of the end of this year, 263 patients in total are under medical management in our outpatient clinic. As shown in the figure, the number of total patients declined in 1997 because a part of patients as well as medical stuffs were moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again with exponential curve 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

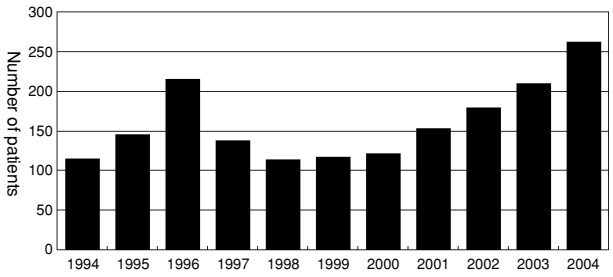

Figure 1. The number of HIV-infected patients in IMSUT hospital

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 research of HIV infection in IMSUT hospital: Specific immune therapy for human immunodeficiency virus followed by structured treatment interruption of antiretroviral therapy (Phase I study).

Great improvement has been achieved in antiretroviral therapy for HIV infection after introduction of highly active antiretroviral therapy (HAART) using three or more anti-HIV drugs. HIV-infected patients now have better survival and frequency of admission for serious opportunistic infection is decreasing. However, it is estimated to take more than 60 years of treatment for eradication of HIV from patients, which means that they have to continue HAART for all over their lives. Long term HAART deteriorates patients Å quality of life and causes adverse effects including metabolic abnormalities.

To overcome these problems, we started a clinical trial to interrupt HAART after specific immune therapy to HIV-infected patients. We previously reported that HIV-specific immunity had quantitative and qualitative abnormality in HIV-infected patients. Consequently, if HAART is stopped, HIV quickly starts to replicate and host immunity cannot stop the proliferation. However, if HIV-specific immunity is recovered by HIV vaccine in patients who are in good viral control under HAART, HIV replication may be partially suppressed by host immunity, even after interruption of HAART, to the level that does not decrease CD4+ T cells. To test this hypothesis, we planned a phase I study: Specific immune therapy for human immunodeficiency virus followed by structured treatment interruption of antiretroviral therapy, where dendritic cells (DC) which are derived from patients and pulsed with HIV epitope peptides are used as HIV vaccine.

Four patients under HAART and with good viral control were enrolled and finished 6 doses of HIV DC vaccine. After patients stopped HAART following vaccination, their viral load increased within 4 weeks to the level that they initiated HAART. Immunological analysis of peripheral blood mononuclear cells revealed that HIV DC vaccine induced HIV-specific immunity in 2 of 4 patients. However, the specific immunity seemed to be too weak to suppress viral proliferation after interruption of HAART. We need to explore more efficient way for induction of HIV-specific immunity.

2. Treatments and Clinical Research of Tropical Diseases

Tetsuya Nakamura, Takashi Odawara[1], Takeshi Fujii[1], Tokiomi Endoh[1], Jun-ichi Takeda[1], Fuyuaki Ide[1], Takeshi Matsumura[1], Hitomi Nakamura[1], Miou Sato[1], Mieko Goto[1] and Aikichi Iwamoto[1]: [1]Department of Infectious Diseases

a. Treatment of Tropical Diseases in IMSUT hospital: Statistical characteristics of HIV-infected patients in IMSUT hospital this year

This year, 132 travellers visited our clinic for consultation or treatment of tropical diseases. Forty-two of 132 visited clinic before travel for prescription of malaria prophylaxis (28 trevallers), vaccination (10 trevallers) and other general consultation (4 travellers). Ninety travellers visited clinic after travel because of sickness, and we diagnosed and treated 16 cases of traveller's diarrhea, 11 post-exposure prophylaxis of rabies, 4 malaria, 3 amoebiac enteritis, 2 typhoid fever, 2 dengue fever, 1 giardiasis, and 1 paratyphoid fever.

b. Clinical research of Tropical Diseases in IMSUT hospital : Questionnaire-based analysis of mefloquine chemoprophylaxis for malaria in Japanese.

In Japan, indigenous malaria has been eradicated since 1961. However, an increasing number of Japanese have visited malaria-endemic countries, and 100-160 cases of imported malaria have been reported annually for these 10 years. Thus, prophylaxis of malaria is an important issue for travelers to endemic areas; especially sub-Saharan Africa and Oceania. Although cau-

tion against mosquito bites is an essential prevention, chemoprophylaxis using anti-malarial drugs is inevitable when travelers stay in highly endemic areas for a substantial period.

Mefloquine is a quinine-related compound with strong anti-malarial activity, and has been proven highly effective for not only treatment of an acute phase of malaria but also its prophylaxis. World Health Organization recommends mefloquine as first choice for chemoprophylaxis in more than 50 malaria-endemic countries. However, side effects of mefloquine, such as nausea, vomiting, dizziness, weakness, sleep disturbance, and dysphoria, are relatively common. The main serious side effect of mefloquine is a neuropsychiatric effect which occurs in 1: 10,000 when used as chemoprophylaxis. In fact, several studies have shown that mefloquine is not well tolerated due to adverse effects.

In Japan, mefloquine was approved in November 2001 for both treatment and prophylaxis of malaria, and is the only licensed drug for malaria chemoprophylaxis as of October 2004. Doxycycline is also commercially available but not licensed for malaria chemoprophylaxis. Thus, health care providers have no choice other than mefloquine when they prescribe malaria chemoprophylaxis. However, mefloquine was approved based on clinical data obtained from a clinical trial in Japanese population for treatment of acute phase of malaria, and no information has been reported as for effects and adverse effects of prophylactic usage of mefloquine in Japanese population. Although several groups in other countries had reported data about mefloquine chemoprophylaxis, information in Japanese population is fundamental for safe and effective chemoprohylaxis because of genetic difference between populations.

We thus performed a questionnaire-based study in 21 travelers who were prescribed mefloquine for malaria chemoprophylaxis from October 2001 to December 2003. The study revealed that only 38.1% of prescribed travelers could complete prophylaxis schedules. 38.5% of travelers with incomplete adherence stated that they did not take mefloquine because of either actually experienced or anticipated adverse effects. In fact, 75.0% of travelers who took mefloquine had at least one of adverse effects related to mefloquine. As an overall impression about mefloquine chemoprophylaxis, 19.0% of travelers stated not to take mefloquine again for the next travel to malaria-endemic areas because of adverse effects. These results suggest that other effective and well-tolerated chemoprophylactic antimalarials must be available for Japanese travelers who are intolerant to mefloquine.

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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 and regenerative medicine, and analysis of pathogenesis of hematopoietic disorders.

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

Hirohide Kawasaki, Yasuhiro Ebihara, Kohichiro Tsuji

Although a standard regimen in hematopoietic stem cell transplantation (SCT) has been available for children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia, it has not been standardized for those with rare diseases including congenital bone marrow failure syndrome (CBMFS) and natural killer cell leukemia. A multi-institutional trial using regimens with a rationale should be proposed in a prospective manner. For CBMFS, we conducted *in vitro* and *in vivo* assays to assess the sensitivity of 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 SCT 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 disease, 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 Philadelphia chromosome-positive acute lymphoblastic leukemia in children

Hirohide Kawasaki, Atsushi manabe[1], Kohichiro Tsuji, Keizo Horibe[2]; [1]Department of Pediatrics, St. Luke's International Hospital, [2]Department of Pediatrics, National Nagoya Hospital

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) continues only 3-5% of ALL in children: however, its prognosis is known to be very poor despite of contemporary multiagent intensive chemotherapy. The meta-analysis of over 300 children with Ph^+ ALL demonstrated the efficacy of allogeneic hematopoietic SCT from matched sibling donor.

Imatinib mesylate was recently produced as a specific tyrosine kinase inhibitor for bcr-abl fusion gene product in chronic myelogeneous leukemia. Because the number of children with Ph^+ ALL is small, a nationwide trial for the disease is mandatory. On behalf of the Japanese Pediatric Leukemia/Lymphoma Study Group, we proposed a trial, which employs intensive chemotherapy and a new drug, imatinib mesylate, to maintain a remmision status, followed by allogeneic SCT at the 8th month after the diagnosis. This is a phase II study to evaluate the efficacy of imatinib mesylate. The efficacy will be assessed with molecular quantification techniques (qualitative and quantitative real-time polymerase chain reaction (PCR) method). The toxicity of the drug will be monitored and graded by the criteria of NCI-CTC VER2.0. The study was permitted by the ethical committee of the Japanese Society of Pediatric Hematology, and opened in 2004.

3. MxA expression as a specific marker for viral infections after allogeneic stem cell transplantation

Atsushi Manabe[1], Hirohide Kawasaki, Yasuhiro Ebihara, Kohichiro Tsuji

Many patients suffer febrile disease soon after allogeneic SCT. The symptoms of viral infections and acute graft-versus-host disease (aGVHD) after allogeneic SCT are similar and often difficult to distinguish. However, an accurate diagnosis is important since the treatments for these diseases are very different. It is known that MxA protein is specifically induced in patients with several viral infections. We investigated the cytoplasmic expression of MxA in the peripheral blood lymphocytes of patients with fever after receiving allogeneic SCT, using a newly generated monoclonal antibody (KM1135) and flow cytometry. The level of MxA expression was significantly higher in patients diagnosed with viral infection than control individuals. The level of MxA in patients with aGVHD was identical to that in controls. Of note, it was demonstrated that the level of MxA well correlated with the amount of the cytomegalovirus antigen-positive cells in the presence of aGVHD. The measurement of MxA is simple and useful in distinguishing viral disease from other conditions including aGVHD after allogeneic SCT.

4. Cooperative clinical trial for pediatric myelodysplastic syndrome

Atsushi Manabe[1], Hirohide Kawasaki, Yuji Zaike[3], Yasuhiro Ebihara, Kohichiro Tsuji; [3]Department of Laboratory Medicine, Research Hospital

Pediatric myelodysplastic syndrome (MDS) is a rare disease, and only 50-100 children under the age of 16 suffer from the disease annually. The diagnosis and treatment 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 200 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.

5. Molecular pathogenesis of pediatric myelodysplastic syndrome and myeloproliferative diseases

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Pediatric MDS and myeloproliferative diseases (MPD) are very rare disorders. The diseases are commonly seen in elderly patients. It suggests that the pathogenesis of the diseases in children be of germline origins rather than of acquired process. In fact, germline mutations have been elucidated in a large proportion of pediatric MDS and MPD: GATA1 mutations in patients with MDS and Down syndrome; FANC mutations in those with MDS and Fanconi anemia; PTPN11 mutations or NF1 mutations in those with JMML.

We also tested the epigenetic abnormalities. Aberrant DNA methylation is frequently observed in adults with MDS, and is recognized as a critical event in the disease's pathogenesis and progression. The frequency of *p15* hypermethylation in pediatric MDS was 78%, which was comparable to that in adult MDS. In contrast, *p15* hypermethylation in JMML was a rare event. In JMML, clinical and laboratory characteristics including *PTPN11* mutations and aberrant colony formation were not different between the patients with hypermethylated *p15* and the oth-

ers. Aberrant methylation of *p16* was not detected in children with either MDS or JMML. Since *p15* and *p16* genes were unmethylated in children with JMML, in whom the disease had progressed with an increase in the number of blasts, a condition referred to as blastic crisis, we infer that the aberrant methylation of these genes is not responsible for the progression of JMML. The result suggests that demethylating agents may be effective in most children with MDS and a few patients with JMML.

6. Molecular pathogenesis of juvenile myelomonocytic leukemia

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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 bone marrow (BM) cells of JMML patients and normal volunteers without and with GM-CSF, respectively, in a dose-dependent manner in clonal culture. At 10 μM 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.

7. Production of hematopoietic stem cells and functinal blood cells from human embryonic stem cells

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Embryonic stem (ES) cells are pluripotent cells derived from preimplantation embryos. Since ES cells have the ability to be maintained in culture indefinitely as undifferentiated cells, yet they are capable of forming more differentiated cell types, human ES cells recently established are expected as a novel source of human transplantable cells. We then planed to produce hematopoietic stem cells for SCT and functional blood cells for transfusion medicine from human ES cells. This study was permitted by the ethical committee of the Japanese Government on December 20, 2003, and started. On beginning this study, we thought that *in vitro* reconstitution of hematopoietic circumstance of embryo is important to induce the differentiation of human ES cells into HSC and functional blood cells. To achieve this, we established stromal cells from embryonic hematopoietic tissues such as aorta-gonad-mesonephros (AGM) region at 10.5 days post coitus (dpc) and fetal liver (FL) at 15.5 dpc of mouse embryos, because long term-repopulating HSC are first generated in AGM region at 10 dpc, and shift to FL in which hematopoiesis dramatically expands. As expected, hematopoietic cells were generated from human ES cells in the coculture with the mouse embryo -derived stromal cells. We are now evaluating the function of the cells differentiated from human ES cells, and searching the molecules contributing to the capability of the stromal cells to induce the differentiation of human ES cells to hematopoietic cells.

8. Production of mesenchymal stem cells from human placenta

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Mesenchymal stem cells (MSC) are widely distributed in a variety of tissues in the adult human body, and are present in the fetal environment. However, MSC are a rare population in these tissues. Then we tried to identify cells with MSC-like potency in human placenta. We isolated adherent cells from trypsin-digested term placentas and established two clones by limiting dilution. We examined these cells for morphology, surface markers, gene expression patterns, and differentiation potential and found that they expressed several stem cell markers, hematopoietic/endothelial cell-related genes,

and organ-specific genes, as determined by reverse transcription-PCR and flow cytometric analysis. They also showed osteogenic and adipogenic differentiation potentials under appropriate conditions. We suggest that placenta-derived cells have multilineage differentiation potential similar to MSC in terms of morphology, cell-surface antigen expression, and gene expression patterns. The placenta may prove to be a useful source of MSC.

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We participate in cutting edge science of autoimmune, rheumatic and allergic diseases and novel treatments for them. In addition to clinical studies aimed to improve the efficacy and safety of current therapies, we are going to carry out experimental protocols of particular interest for patients not responding to conventional therapy. For these purposes, we have vigorously been collaborating with the Division of Clinical Immunology (Prof. Chikao Morimoto).

I. Therapeutically targetting transcription factors

Noritada Yoshikawa, Hirotoshi Tanaka, Rheumatology Clinic / Department of Rheumatology and Allergic Diseases, Noriaki Shimizu, Hiroshi Nakamura, Kensaku Okamoto, Tetsuya Hisada, Chikao Morimoto / Division of Clinical Immunology

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

a. Glucocorticoid receptor project

Glucocorticoid hormones are effective in controlling inflammation, but the mechanisms that confer this action are largely unknown. It has been shown that both positive and negative regulation of gene expression are necessary for this process. The genes whose activity is negatively modulated 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 glucocorticoid receptor (GR), but have instead regulatory sequences for transcription factors such as AP-1 or NF-κB. Considering various severe side effects of glucocorticoids, it may be pharmacologically important to dissociate these negative regulatory function of the GR from induction of metabolic enzymes, gene expression of which has been shown to be positively regulated by the GR. We propose that a certain class of compounds (surprisingly, some of them are 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 the strategy for identification of novel therapeutic strategy.

(i) Development of dissociating ligand for the glucocorticoid receptor

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

On the other hand, receptor specificity is another important aspect of novel GR regulator. In this line, we have shown that cortivazol is extremely specific for GR and does not bind to MR. In the course of studying the molecular basis for this receptor specificity of the ligand using cortivazol as a model, we have clarified that the ligand binding domain of the GR modulates gene transcription in concert with its own DNA binding domain in a ligand-dependent manner. This intrareceptor communication clearly adds new dimension for further consideration of the dissociated ligands.

(ii) Molecular biology and clinical application of a novel protein HEXIM1

HEXIM1 is a nuclear protein, expression of which is induced by treatment of vascular smooth muscle cells with a differentiation inducer hexamethylane bisacetamide. Recently, we have found that HEXIM1 participates in transcriptional regulation in various fashion. For example, HEXIM1 interacts with those factors related to basal transcription and splicing, mainly via binding with small nuclear RNA. Moreover, HEXIM1 interferes GR action via locking GR into a distinct compartment in the nucleus. These studies will open up a door for novel therapy for a variety of diseases.

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

HIF-1α is essential for not only angiogenesis but also maintenance of a variety of physiological processes. In this line, molecular biology of HIF-1α will provide us possible advantage to characterize and manupilate such processes. Angiogenesis is regulated by a combination of variety factors including transcription factors. We have recently identified that HIF-1α function is regulated in a various fashion in certain physiological settings, which may be important of homeostatic control of tissue function. In this line, we are now identifying the molecular mechanism for such regulation of HIF-1α. Among others, we clearly showed that in human peripheral T cells expression of HIF-1α is controlled by not only oxygen tension but also T cell receptor-mediated signals. Especially, involvement pf PI3 kinase and mTOR is critical. Moreover, treatment of T cells with rapamycin inhibits expression of HIF-1α protein and its target genes. Currently we are working with the molecular mechanism of HIF-1α expression and its biological significance.

II. Characterization of the tetraspanin CD9 on human CD4+CD45RA+ Naive T cells

Osamu Hosono, Hirotoshi Tanaka, Rheumatology Clinic / Department of Rheumatology and Allergic Diseases, Hiroshi Kobayashi, Satoshi Iwata, Chikao Morimoto, Division of Clinical Immunology

Human $CD4^+$ T cells can be divided into reciprocal memory and naive T cell subsets based on their expression of CD45 isoforms and CD29 /integrin beta1 subunit. To identify unique cell surface molecules on human T cells, we developed a new monoclonal antibody termed anti-5 H9. Binding of anti-5H9 triggers a costimulatory response in human peripheral blood T cells. Retrovirus-mediated expression cloning has revealed that the antigen recognized by anti -5H9 is identical to the tetraspanin CD9. We now show that human CD9 is preferentially expressed on the $CD4^+CD45RA^+$ naive T cell subset, and that $CD9^+CD45RA^+$ T cells preferentially respond to the recombinant $beta_2$-glycoprotein I, as compared to $CD9^-CD45RA^+$ T cells. Furthermore, anti-5H9 inhibits both the recombinant $beta_2$-glycoprotein I- and the recall antigen tetanus toxoid-specific T cell proliferation. These results suggest that the tetraspanin CD9 plays an important role in T cell activation.

III. Roxithromycin specifically inhibits development of collagen-induced arthritis

Osamu Hosono, Hirotoshi Tanaka, Rheumatology Clinic / Department of Rheumatology and Allergic Diseases, Yasuyo Urasaki, Mamoru Nori, Satoshi Iwata, Chikao Morimoto, Division of Clinical Immunology

Roxithromycin (RXM) is a macrolide antibiotic that is effective in the treatment of chronic lower respiratory tract diseases including diffuse pan-

bronchiolitis and bronchial asthma. However, its mechanism of action apart from its antibacterial action remains unclear. To further determine the mechanism of action of RXM, we attempted to evaluate the effect of RXM on T cell functions and the inflammatory responses in mice with collagen-induced arthritis (CIA).

RXM did not affect the production of Th1-type and Th2-type cytokines, whereas it specifically inhibited production of such proinflammatory cytokines as TNF- and IL-6 by T cells as well as macrophages. In addition, RXM inhibited T cell migration. Moreover, it was shown that RXM treatment of CIA mice reduced the severity of arthritis and serum level of IL-6, as well as leukocyte migration into the affected joints and destruction of bone and cartilage. Our findings strongly suggest that RXM may be useful for the therapy of rheumatoid arthritis.

IV. Study on serum soluble CD26 in patients with immune-mediated diseases

Osamu Hosono, Yuichi Makino, Noritada Yoshikawa, Hirotoshi Tanaka, Rheumatology Clinic / Department of Rheumatology and Allergic Diseases, Kei Ohnuma, Chikao Morimoto, et al., Division of Clinical Immunology

CD26 is the cell surface activation antigen with dipeptidyl peptidase IV (DPPIV) enzyme activity that is preferentially expressed on memory T cells and has a role in T cell immune responses. The soluble form of CD26 is present in serum and recombinant soluble CD26 can enhance in vitro antigen-specific T cell responses. Serum levels of sCD26 and its specific DPPIV activity were significantly decreased in SLE and were inversely correlated with SLE disease activity index score, but not with clinical variables or clinical subsets of SLE. 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 have also measured sCD26/DPPIV levels in sera from patients with RA and found significant decrease of sCD26 and its specific DPPIV activity. We plan to examine the effect of infliximab (anti-TNFa monoclonal antibody) therapy on serum levels of sCD26/DPPIV in patients with RA.

V. Immunobiology and clinical applications of innate and acquired immune systems.

Hiroshi Kawasaki, Rheumatology Clinic / Department of Rheumatology and Allergic Diseases, and Chikao Morimoto, Division of Clinical Immunology (in collaboration with Katsuaki Sato, Takami Matsuyama, and Kouichi Hirai)

We have been pursuing the structure and functional analysis of human proinflamatory cytokines and their receptor system in order to clearly address their roles in innate and acquired immune system. Molecules of our interest in this field at present are IL-12, IL-23 and TRAIL.

a. Structure and function of IL-12 receptors and IL-23 receptors.

Human CD4 T cells respond to antigenic stimuli to develop in two ways, Th1 and Th2 phenotypes. Th1-type CD4 T cells produce Interferon gamma to promote cellular immunity and Th2-type CD4 T cells produce IL-4 to promote humoral immunity. IL-12 is the key cytokine that preferentially lead antigen-stimulated T cells to mature into Th1-type. We previously analyzed the IL-12 receptors expressed in activated T cells with the aid of monoclonal antibodies elaborated in our hands against IL-12 receptor beta1 chain. In the course of the work, we verified the association of the beta 1 chain with the putative beta 2 chain., which was in line with the proposed structural model of IL-12 receptor system. Surprisingly, we identified a 75-Kd phosphorylated molecule that formed complex with IL-12 receptor beta 1 chain. Recently, IL-23 was identified and the IL-23 receptor was shown to form a dimer with the IL-12 beta 1 chain. We are in the process of newly establishing monoclonal antibodies raised against the IL-23 receptor to elucidate the identity of our 75-Kd molecule.

b. The Role of TRAIL in the prevention of Acute Graft-Versus-Host Dosease.

We report here the potential usefulness of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) for the treatment of lethal acute graft-versus-host disease (GVHD) and leukemia relapse. Dendritic cells (DCs) genetically modified to express TRAIL showed more potent cytotoxicity than soluble TRAIL against both alloreactive T cells and leukemic cells mediated through TRAIL/death receptor (DR) pathway. In addition, cell gene therapy with genetically modified DCs expressing TRAIL was more effective than in vivo gene transfer of TRAIL for the protection against acute GVHD and leukemia relapse. Thus, gene transfer of TRAIL involving DCs is useful for the treatment of acute GVHD and leukemia relapse by selective targeting of the pathogenic T cells and leukemia relapse.

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Department of Advanced Medical Science was established in September 1997. We are investigating, (1) Analysis on mechanisms of conotruncus formation during embryonic heart development, (2) Identification of a tumor-associated antigen targeted by the dendritic cell therapy, (3) Analysis of the expression gradient of genes in human colonic mucosa, (4) A potential pro-angiogenic cell therapy for ischemic disease using human placenta-derived cells. We are planning and progressing several projects described below to develop a new therapy for several diseases, including cancer and ischemic disorders.

Analysis on mechanisms of conotruncus formation during embryonic heart development

Nakaoka T. et al.

The heart defect (hdf) mouse is a recessive lethal mutation that arose from a LacZ reporter containing a transgene insertional mutation. Embryos homozygous for the transgene die at approximately embryonic day (E) 10.5 due to the cardiac anomaly. The portion of the heart, comprising the left ventricle and atrium, in hdf homozygous embryos are highly dilated with the intervening atrioventricular endocardial cushions absent. The most striking feature of the hdf homozygous embryo is the immature conotruncal outlet segment, through which diminutive right ventricle connects directly to the aortic arches. Therefore, the hdf mouse is a good model system to investigate the molecular pathways that lead to the accretion of a conotruncal outlet segment to the simple tubular heart at its arterial pole. In order to address molecular mechanisms responsive for defective heart development in the hdf mouse, we performed subtractive hybridization and consequently, a novel gene, Hag2 (hdf affected gene 2) was identified as a gene whose expression is affected in the hdf mouse. Hag2 was a single exon gene of some 8 kb in length located on mouse chromosome 1. in vitro translation of the capped transcript of Hag2 produced a single polypeptide of 17 kDa with a predicted open reading frame of 156 amino acids not homologous to the other reported proteins. Hag2 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 Hag2 expression was clearly detected in neuroepithelium, the pharyngeal arches, limb buds and somites. Hag2 expression in the pharyngeal arches correlated temporally with the passage of neural crest cells and recruitment of the cells from anterior heart field to the outflow tract. Thus, the dynamic expression pattern of Hag2 in the pharyngeal arches suggests its potential involvement in the

defective formation of the outflow tract observed in the hdf mouse.

Identification of a tumor-associated antigen targeted by the dendritic cell therapy

Yoshiura K. et al.

Tumor-associated antigens (TAAs) are promising candidates for target molecules in the immunotherapy and a wide variety of TAAs have been discovered by the presence of serum antibodies in cancer patients. We previously conducted the dendritic cell (DC) therapy on 10 malignant melanoma patients and massive tumor necrosis occurred in two patients. In this study, we found a 29 kD protein of which antibody was elicited by the DC therapy in one patient with the tumor necrosis. Matrix-Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry analysis of the protein isolated by two-dimension electrophoresis combined with Western blots revealed that the 29 kD protein is carbonic anhydrase (CA)-II. Immunohistochemistry of the tumor and normal tissues showed that CA-II was expressed in the tumor vessel but not in normal vessel endothelium. CA-II expression in tumor endothelium was observed in other cancers including esophageal, renal and lung cancers as well. In an *in vitro* angiogenesis model, CA-II expression of normal human vein endothelial cells was significantly up-regulated when cells were cultured in the acidic and hypoxic condition which mimics a tumor environment. These findings suggest that CA-II is a tumor vessel endothelium-associated antigen in melanoma and other cancers, and anti-CA-II immunity elicited by the DC therapy may be associated with tumor vessel damage causing tumor reduction.

Analysis of the expression gradient of genes in human colonic mucosa

Ohno H. et al.

Ulcerative colitis 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 expression gradient of genes in human colonic mucosa might be related to the disease development and progression. We compared the expression levels of genes in segments of a normal human colon and made a catalogue of genes expressed 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 studies were conducted to confirm the expression levels. Finally, we evaluated the expression levels of these genes throughout the digestive tract and other tissues by northern blotting studies. 3 genes showed gradual rise in expression in colon and one of them was specifically expressed in colon. These genes might be susceptible for ulcerative colitis.

A potential pro-angiogenic cell therapy for ischemic disease using hPDMCs

Nishishita T. et al.

After the establishment of Cord Blood Banks, more than 2,000 cord blood transplantations have been performed throughout the world. In the processing of cord blood, adjacent placenta has been so far thrown away. Recently, the Department of Cell Processing IMS, started preparation and characterization of human placenta-derived mesenchymal cells (hPDMCs), which are obtained from placental villi. One of the characteristics of placenta is that its high vascularity. So, in our laboratory, we explored the possibility that these cells might produce angiogenic cytokines and could be used for pro-angiogenic cell therapy. We measured VEGF in hPDMCs conditioned media by ELISA and found that a large amount of VEGF, comparable to the amount produced by cancer cells, is produced by hPDMCs. We confirmed this VEGF is biologically active.

In vivo studies were performed to test the efficacy of hPDMCs injection to improve ischemic status. We made an animal model for arterial occlusive disease, inducing unilateral hindlimb ischemia by binding the left femoral arteries and veins of NOD/Shi-scid mice. We transplanted hPDMCs in the ischemic muscles. Subcutaneous blood perfusion was analyzed by using a laser doppler perfusion image analyzer before and after transplantation. Transplantation of hPDMCs significantly improved the blood flow of the affected limbs. In the limbs of treated mice formation of blood vessels was more prominently observed as compared to the control. Transplanted hPDMCs produced hVEGF for at least 7 days in NOD/Shi-scid mice, which was demonstrated by real time RT-PCR. It was concluded that hPDMCs could be used to treat human ischemic diseases.

Angiogenesis in placenta is different from angiogenesis by malignant tumors in that mature non-leaky vessels are formed. We evaluated other angiogenic factors than VEGF. We detected large amount of bFGF in hPDMCs' cell

lysate. We also detected much larger amount of angiopoietin-1 and angiopoietin-2 mRNA in hPDMCs than control tumor cells. We assume that the presence of these multiple angiogenic factors in combination contribute to the formation of mature blood vessels.

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

1. Summary of surgical treatment and other procedures performed in 2004

Hideaki Tahara, Takuya Tsunoda, Yoshifumi Beck, Akihiko Itoh, Yasutaka Takeda, Akihiko Takeda, Takuya Takayama, Yuichi Ando, Hiroaki Uchida, Hiroyuki Mushiake, Naoya Ichikawa, Juichiro Konisi, Koji Yoshida, Hajime Ishikawa, Norihiro Kokudo[1], Masatoshi Makuuchi[1] : [1]Division of Hepato-Biliary-Pancreatic Surgery and Artificial Organ and Transplantation, Department of Surgery, Graduate School of Medicine, University of Tokyo, Japan.

Surgical operations have been performed in 118 cases under general anesthesia and spinal or epidural and/or local anesthesia. As shown in Table 1, major operations were performed in 71 patients with malignant diseases and in 35 patients with benign diseases.

Procedures other than surgical operations performed in 2004 were as follows: gastroduodenal endoscopy (391 cases), and colorectal endoscopy (179 cases).

Table 1. Major operations performed in 2004

Malignant Diseases		Benign Diseases	
Cancer of the stomach	8	Cholelithiasis	11
Cancer of the colo-rectum	18	Inguinal hernia	7
Cancer of the liver	8	Miscellaneous	17
Cancer of the pancreas	2	Total	35
Cancer of the breast	16		
Cancer of the thyroid	6		
Miscellaneous	13		
Total	71		

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

Takuya Tsunoda, Takuya Takayama, Akihiko Itoh, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Hirouki Mushiake, Hiroaki Uchida, Kohji Yoshida, Hajime Ishikawa, Hideaki Tahara

Phase I clinical trial has been performed to evaluate safety, immunological response and clinical response against advanced malignant melanoma patients. Epitope peptides derived from gp100, a melanoma associated antigen, are used for the cancer vaccine to treat the patients with advanced malignant melanoma. Patients with HLA-A*0201 were treated with a gp100 derived peptide (ITDQVPFSV) and another peptide with a mutation (IMDQVPFSV). All of the peptides were used with incomplete Freund's adjuvant (IFA) in order to augment for anti-tumor immunity. So far, five patients with stage IV melanoma have been immunized with a vaccine consisting of HLA-A*0201-restricted epitope peptide derived from gp100 melanoma differential antigen emulsified with IFA. No adverse effects without grade I toxicity were observed in these patients. Immunological monitoring was performed to determine IFN-g production using PBMC stimulated with the vaccinated peptides. Immunological response and clinical response have not been obtained so far. We continue to perform the immunological response.

3. Phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides restricted to HLA-A*2402

Takuya Tsunoda, Takuya Takayama, Akihiko Itoh, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Hirouki Mushiake, Hiroaki Uchida, Kohji Yoshida, Hajime Ishikawa, Hideaki Tahara

Epitope peptides derived from gp100, a melanoma associated antigen, are used for the cancer vaccine to treat the patients with advanced malignant melanoma. We have performed phase I clinical trial that six patients with stage IV melanoma were immunized with a vaccine consisting of HLA-A*2402-restricted epitope peptide derived from gp100 melanoma differential antigen (gp100-int4: VYFFLPDHL) emulsified with incomplete Freund's adjuvant (IFA). No adverse effects without grade I toxicity were observed in these patients. Patient 1 had a partial regression of multiple liver metastases and decrease of tumor maker after vaccination. In two patients (Patient 2 and 3), vitiligo was observed after vaccination.

From phase I data, phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides were performed. HLA-A*2402-restricted gp100 derived peptide (gp100-int4) was used with IFA and interleukin (IL-2) in order to augment for anti-tumor immunity. Our goals in this clinical trial are to examine these clinical efficacy, furthermore, safety and immune responses associated with the peptide vaccination. We have enrolled 13 melanoma patients during year 2003. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted. Patient 9 had a stable disease of multiple lung metastases for 17 months since the first vaccination. In two patients (Patient 8 and 9), vitiligo was observed after vaccination. Immunological monitoring was performed to determine IFN-g production and analyze A24/gp100 tetramer staining using PBMC stimulated with gp100-int4 peptide. PBMC from Patient 9 was determined significant amount of IFN-g production and specific reactivity of IFN-g production to gp100-int4 peptide after vaccination. By A24/gp100 tetramer analysis, A24/gp100 tetramer and CD8 double positive subset was detected after vaccination in PBMC from Patient 9. Furthermore, melanoma-specific CTLs were established from CD8 and A24/gp100 tetramer double positive subset in this patient. Importantly, these CTLs were able to lyse 888mel (HLA-A24 positive and naturally expressing gp 100), but not to lyse 397mel (HLA-A24 negative and naturally expressing gp100) and HT29 (HLA-A24 positive and gp100 negative). It might be of significance that not only HLA-tetramer but also IFN-g production were necessary to evaluate immunological response induced by peptide-based vaccine.

4. Phase I/IIa clinical trial of epitope peptides based vaccine against gastrointestinal malignancy

Takuya Tsunoda, Takuya Takayama, Akihiko Itoh, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Hirouki Mushiake, Hiroaki Uchida, Kohji Yoshida, Hajime Ishikawa, Hideaki Tahara

Epitope peptides derived from MAGE3 and HER2/neu are used for the cancer vaccine to treat the patients with advanced gastrointestinal malignancy. Patients with HLA-A*0201 were treated with MAGE3 and HER2/neu derived peptide (FLWGPRALV, KIFGSLAFL). Patients with HLA-A*2402, were treated with MAGE3 and HER2/neu derived peptide (IMPKAGLLI, RWGLLLALL). All of the peptides were used with IFA and IL-2 in order to augment for anti-tumor immunity. To analyze the immune response of the vaccinated patients, HLA-

Tetramer was prepared and used for staining of the peripheral blood lymphocytes taken from the patients enrolled in this protocol. Our goals in this clinical trial are to examine these clinical efficacy, furthermore, safety and immune responses associated with the peptide vaccination. We have enrolled 1 esophageal cancer patient for MAGE3-HLA-A*2402 peptide until now. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted.

5. Phase I clinical trial of epitope peptides based vaccine with novel tumor associate antigen, RNF43, found by genome-wide exploration using cDNA Microarray Profiling (GET-MAP) against colorectal cancer patients.

Takuya Tsunoda, Marimo Sato, Takuya Takayama, Akihiko Itoh, Yoshifumi Beck, Hiroaki Tanaka Juichiro Konishi, Hirouki Mushiake, Hiroaki Uchida, Kohji Yoshida, Hajime Ishikawa, Hideaki Tahara

We have performed genome-wide exploration using cDNA Microarray Profiling, and successfully identified a new tumor-associated antigen (TAA) which can induce potent cytotoxic T-cells (CTLs) specific to tumor cells. In our preceding study, we identified multiple new genes using gene expression profiling with a genome-wild cDNA microarray containing 23040 genes. Among them, we selected RNF43 (Ring Finger Protein 43) as a promising candidate for a TAA expressed by colon cancer cells. We examined in this study whether the RNF43 protein contains antigenic epitope peptides restricted to HLA-A *0201 or HLA-A*2402. The CTL clones were successfully induced with stimulation using the peptides binding to HLA-A*0201 (ALWPWLLMA and ALWPWLLMAT) and HLA-A*2402 (NSQPVWLCL), and these CTL clones showed the cytotoxic activity specific to not only the peptide-pulsed targets but also the tumor cells expressing RNF43 and respective HLAs. These results strongly suggest that RNF43 is a new TAA of colon cancer. Furthermore, these results also suggest that our strategy might be a promising one to efficiently discover clinically useful TAAs.

From these basic results, phase I clinical trial has been performed to evaluate safety, immunological response and clinical response against advanced colorectal cancer patients. Epitope peptides derived from RNF43 are used for the cancer vaccine to treat the patients with advanced colorectal cancer. Patients with HLA-A *0201 were treated with RNF43 derived peptide (ALWPWLLMAT). Patients with HLA-A*2402, were treated with RNF43 derived peptide (NSQPVWLCL). All of the peptides were used with IFA in order to augment for anti-tumor immunity. Five patients with HLA-A*2402 have been enrolled, and one patient with HLA-A *0201 has been enrolled. We perform to analyze the immunological response using HLA tetramer and the specific INF-g production.

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Our department consists of three major divisions: diagnostic radiology, nuclear medicine and radiation oncology. Diagnostic radiology plays a crucial role in evaluating various neoplastic and infectious diseases. Researches aim at sophisticating diagnostic procedures in clinical practice. In nuclear medicine, we develop analytic methods to estimate in vivo physiology, as well as studying the tracer kinetics and physical characteristics of detectors. Total body irradiation prior to bone marrow transplantation is a major role of our division of radiation oncology.

Diffusion-weighted and diffusion tensor imaging of the human spinal cord

Toshiyuki Okubo

Echo-planar diffusion-weighted Imaging (EP-DWI) and diffusion tensor imaging (DTI) is a advantageous method for early detection of cerebral ischemia. EP-DWI and DTI offers multisectional images sensitive to cytotoxic edema in a very short aquisition time and is almost free from motion artifact. EP-DWI is applied to other cerebral disorders such as degenerative disease, demyelinating disease, infectios diseas, tumors or so. However, the application of EP-DWI and DTI for spinal cord lesion is limited, because the susceptibility artifacts and low spatial resolution of EP-DWI must be improved. We employ parallel imaging method to reduce the susceptibility artifacts and make a pilot study in which traces of diffusion tensor, fractional anisotropy indices are measured using MR diffusion tensor imaging (DTI) of the spinal cord. The data were obtained with a 1.5-T MRI system. A circularly polarized body coil or head coil was used for both RF transmission and reception of the NMR signal. We use single-shot spin-echo echo-planar sequences (TR/TE＝5000/102msec, 4mm slice thickness and 0.4mm gap, FOV＝28－32×14－16cm^2, NEX＝4, 132×64 pixel matrix, imaging time＝3min 44sec) for diffusion tensor analysis. Diffusion gradients (b-value of 500 s/mm^2 per axis) are always applied on two axes simultaneously around the 180degree pulse. Diffusion properties was measured along 6 noncollinear directions: (Gx, Gy, Gz)＝[(0, 0, 0), (1, 0, 1), (－1, 0, l), (0, 1, l), (0, 1, －1). (1, 1, 0), (－1, 1, 0)]. The six elements of the diffusion tensor D were estimated in each voxel using multivariate regression, and the eigen values (k.) were determined. Maps of Trace (D)/3 (T/3), and the fractional anisotropy (FA) are generated on a voxel-by-voxel basis. Values for T/3, and FA were determined in regions of the cervical cord lesion, normal cervical cord, cerebellum, pons, and CSF by setting region of interest (ROI) on the console of the workstation. The FA of the normal cervical cord is (0.74＋－0.096 mm^2/sec; mean＋－SD). This preliminary study has shown that it is possible to obtain DTI of the human spinal cord in vivo in the short imaging time that is enough to be applied to clinical use. Subsequent improvements in the spatial resolution and reliability of this technique may

lead to comprehend the structural characteristics of spinal cord disease in vivo.

Noninvasive evaluation of therapeutic effects on animal models of acute myeloid leukemia by bioluminescence imaging and magnetic resonance imaging

Yusuke Inoue and Arinobu Tojo[1]: [1]Division of Molecular Therapy, Advanced Clinical Research Center

Imaging technology is increasingly applied to experiments with small animals. Noninvasive imaging permits long-term, repetitive observation of a given animal and is expected to contribute to improve the efficiency and reliability of experiments. We intend to assess therapeutic effect on murine models of Philadelphia chromosone-positive acute myeloid leukemia by bioluminescence imaging and magnetic resonance (MR) imaging. We have established cell lines stably expressing luciferase and transduced with wild-type or mutant BCR/ABL gene and confirmed that expression of luciferase reflects proliferation and therapeutic effect in vitro. The following steps are to implant the cells in mice and to examine disease progression and improvement after effective therapy by using a sensitive CCD camera system. In addition, we are sophisticating methods to image living mice with a low-magnetic-field compact MR scanner. Bioluminescence imaging will offer informations about the amount of viable tumor cells, and MR imaging will show the morphologies of internal organs and tumor masses. We suppose that the two imaging techniques will play complementary roles in assessing temporal changes of mice implanted with tumor cells.

Assessment of cardiac function by magnetic resonance imaging

Yusuke Inoue and Yukihiro Nomura

Magnetic resonance (MR) imaging has been established as an accurate tool to measure the function of the left ventricle (LV). However, it is rather time-consuming and troublesome in view of patient preparation, image acquisition, and data analysis, and is not widely used in clinical practice. We are attempting to solve technical problems in measuring cardiac function by MR imaging. We investigated the applicability of Fourier fitting to the MR evaluation of LV function. An LV time-volume curve was generated from cine cardiac MR images. Fourier fitting was applied to the original curve using one to ten harmonics, and the qualities of the time-volume curve and first-derivative curve were evaluated. LV functional parameters were calculated from the curves generated with and without fitting. The quality of the original time-volume curve was good, and Fourier fitting had no substantial effect on the functional parameters, such as ejection fraction, obtained directly from the time-volume curve. The first-derivative curve generated without fitting showed substantial unphysiological fluctuation. The application of Fourier fitting depressed the fluctuation and tended to decrease estimates of peak ejection rate and peak filling rate. Five or six harmonics appeared to be appropriate for obtaining a high-quality first-derivative curve. Fourier fitting was indicated to aid in reducing unphysiological fluctuation of the first-derivative curve generated from cine cardiac MR imaging and to contribute to evaluation of the functional parameters derived from the first-derivative curve. Our next plan is to sophisticate the determination of the most basal portion of the LV cavity. Our final goals include development of a semiautomatic method of the LV demarcation and that of a four-dimensional display of the cardiac motion.

Therapeutic effect on myocardial flow reserve in metabolic diseases.

Yusuke Inoue and Ikuo Yokoyama[2]: [2]Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo

Myocardial flow reserve can be assessed by rest and stress myocardial blood flow (MBF) measurement using positron emission tomography (PET) and reflects early damage of coronary vessels. Impairment of coronary circulation is essential in managing patients with metabolic diseases, such as hyperlipidemia, diabetes mellitus, and hypertension. We have elucidated decrease in myocardial flow reserve (MFR) in hypercholesterolemia, hypertriglyceridemia, and type II diabetes mellitus in the absence of overt coronary stenosis. We have also demonstrated improvement of MFR after lipid-lowering therapy. As a next step, we attempted to clarify whether reduced MFR in type II diabetics can be improved by an improvement of hyperglycemia. Thirty-two type II diabetics and 17 controls were studied. Myocardial segments which were perfused by angiographically normal coronary arteries were studied. Rest and stress MBF were measured using PET, after which MFR was calculated. Although rest MBF was comparable between type II diabetics and controls, stress MBF was significantly lower in type II diabetics than in controls, as was the MFR. In the patients,

follow-up PET was undertaken after treatment of 6-12 months duration. In 23 patients to whom additional intensive treatment of hyperglycemia was undertaken, MFR was significantly improved. In 9 patients in whom additional treatment of diabetes refused to be undertaken, MFR tended to be worsened. In 14 patients with improved glycemic control, MFR was significantly improved. However, such improvement of MFR did not occur in those with non-responder. Significant inverse relationships were noted between percent change of MFR and percent change of hemoglobin A1c and plasma fasting glucose concentration, respectively.

Assessment of regional insulin resistance by ^{18}F-FDG PET

Yusuke Inoue and Ikuo Yokoyama[2]: [2]Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo

Skeletal muscle glucose utilization (SMGU) during hyperinsulinemic euglycemic clamping can be measured by dynamic PET imaging with ^{18}F-fluorodeoxy-glucose (FDG) to characterize insulin resistance. PET method has a potential to evaluate regional differences in insulin resistance. Dynamic PET for 30-60 min combined with frequent arterial blood sampling is commonly performed to measure SMGU. We have developed simple methods to evaluate SMGU by static PET or dynamic PET without arterial blood sampling. Using the method, we measured SMGU during hyperinsulinemic euglycemic clamping in 22 normotensive type II diabetics on dietary therapy, 17 normotensive non-diabetic hypertriglyceridemics, 22 patients with hypertension, and 12 controls. SMGU was significantly reduced in the three patient groups compared with controls, and the ability of the simplified method to demonstrate regional resistance was proven.

Quantitative analysis of diaphragmatic motion: Dynamic MRI in different postures

Shigeru Kiryu and Masaya Takahashi[3], et.al.: [3]Department of Radiology, Harvard Medical School

It is well known that pulmonary ventilation is altered in different postures, being greater in the dependent lung where gravity is thought to play an important role. However, there have been few studies of lung motion in different postures. We have assessed the relation between right and left hemidiaphragmatic motion during breathing by dynamic magnetic resonance (MR) imaging in normal subjects, and to investigate the alterations in lung motion with changes in posture. Eight healthy subjects were instructed to breathe from end-inspiration to end-expiration as slowly and deeply as possible. Imaging sequences were performed in supine, prone, and left and right lateral decubitus postures. The component of movement of the most cephalic point in the cephalo-caudal axis was measured and the diaphragmatic excursion (maximum hemidiaphragmatic displacement), the synchrony, and the velocity of right and left hemidiaphragmatic motions were calculated in expiratory and inspiratory phases, respectively. Excursion was greater in the right hemidiaphragm in most postures except the left lateral decubitus. In supine and prone postures, both hemidiaphragms moved synchronously in both inspiratory and expiratory phases. In both lateral decubitus postures, the hemidiaphragms moved asynchronously with different velocities in the expiratory phase but with the same velocities in the inspiratory phase. The method described here allowed the assessment of diaphragmatic motions. Motions in the right and left hemidiaphragms changed with posture. In addition, diaphragmatic motion differed between expiratory and inspiratory phases. This study suggests further potential of dynamic MRI for evaluation of pulmonary functions or deficiencies.

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Research Hospital

Department of Laboratory Medicine
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Associate Professor Naoki Oyaizu, M.D., Ph.D.

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Our department consists of seven subdivisions of clinical physiology, hematology, serology, biochemistry, molecular analysis, bacteriology and pathology, and engages in laboratory analysis and diagnosis of clinical materials submitted from the hospital. Since our department is constructed to examine clinical samples on routine bases, our scientific output is currently quite limited. However, along with the ongoing practice of translational research projects in the research hospital, we are now under reconstruction process to evolve into function as an integrated diagnosis & monitoring laboratory.

General Scheme

Our basic research strategy is to characterize molecular mechanisms underlying pathology, develop a way to measure this in the clinical materials or in human disease *in situ.* In particular, developing molecular-based laboratory assay, which enable to assess effectiveness of the experimental clinical trials, is our urgent target. We believe that such an approach is indispensable to direct experimental medicine in a correct way as well as to promote translational research. Developing molecular-based assays in clinical materials requires expertise in pathology and molecular biology; we are thus focusing our specialty to achieve this goal.

1. Pathological evaluation of cancer immunotherapy

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

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 implicate 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 department of hematology, we are going to elucidate molecular mechanisms on the ground of pathology thereby establish new disease entity and develop new therapeutic interventions.

3. Molecular analysis of the chimeric gene expression of hematological disorder

We have initiated to analyze *BCR/ABL* gene expression in specimen from patients with CML and Ph1+ve ALL by real-time PCR and nested RT-PCR techniques. In selected materials, we sequenced the amplified products to provide information for the molecular resistance to STI571 treatment. Now we are expanding target molecules, which include *AML1/ATG8, PML/PAR*α, and *TEL/AML1.*

4. Developing quick & inclusive diagnosis system for infections disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions altered drastically and came into aspects. This results in altered recognition and molecular interaction of infected cells with immune cells, which leads to atypical pathological as well as clinical manifestations. To distinguish infectious disease and immunological disorder is a critical issue, however as a result of modified manifestations, it is a difficult to achieve this in some occasions. To circumvent this, we are pursuing to establish quick and inclusive diagnosis system of infectious disease.

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

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

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助 手 医学博士 高橋直之

Our department was established in April, 2001 to support the translational researches of our hospital. We are participating in the clinical study to examine the SNPs in genes responsible for metabolism of medicines used for hematological malignancies. We are also conducting researches to find responsible SNPs in genes related to drug sensitivity, disease progression, and prognosis in various hematological disorders. In December, 2002 we opened a clinic of genetic counseling in collaboration with the divisions of pediatrics, genetic diagnosis, nursing and so on.

1. Genetic study on CML

Naoyuki Takahashi and Noriharu Sato

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

2. Genetic study on MDS

Naoyuki Takahashi and Noriharu Sato

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

3. Genetic study on GVHD

Naoyuki Takahashi and Noriharu Sato

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

4. Genetic study concerning NK cell receptors and ligands.

Naoyuki Takahashi and Noriharu Sato

Natural killer (NK) cells have gained an increasing concern from hematologists since a re-

port that allogeneic transplantation between killer immunoglobulin-like receptor (KIR)-ligand mismatch pairs has remarkably favorable outcome in acute myeloid leukemia. We have started to search for new alleles for MICB gene and found a new allele in intron 4.

5. Genetic counseling and related activities.

Naoyuki Takahashi and Noriharu Sato

At the genetic counseling clinic, we have seen clients who are suffering or who have family members suffering from genetic diseases. Genetic diseases seen at our clinic this year include neurofibromatosis, G6PD deficiency, osteogenesis imperfecta, Down's syndrome, hemophilia, and spinocerebellar ataxia.

As an initial step to perform individualized medicine, Human Genome Center has started microarray analysis of leukemic cells and lung cancer to predict drug sensitivity. We have also participated in this project concerning patients' selection, informed consents, and notification of the test results.

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Research Hospital

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

Professor	Aikichi Iwamoto, M.D., D.M.Sc.		教　授(併)	医学博士	岩　本　愛　吉
COE Lecturer	Fumitaka Nagamura, M.D., D.M.Sc		特任講師	医学博士	長　村　文　孝
COE Clinical Associate	Seiichiro Kobayashi, M.D., D.M.Sc		特任助手	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 divided into two areas. One is the risk management of research hospital, and the other is the support/monitoring of clinical studies. The staffs of DCTSM (doctors and nurses) are doing their work in collaboration with Translational Research Coordinators (TRC), which are organized by co-medical staffs, including pharmacist, dietician, psychologist and clinical laboratory technologist. The aim of DCTSM is to carry out the safe and ethically-protected clinical trials in the Research Hospital in addition to the data management. In order to accomplish them we are doing the following activities.

Risk management of Research Hospital

Fumitaka Nagamura, Seiichirou Kobayashi, Mitsui Kobayashi, Aikichi Iwamoto

The occurrence of medical accidents is the major problem at the hospitals. The requirement to avoid and to reduce the accidents has been increasing year by year. The aim of Research Hospital is to promote the translational researches, and reliance of the Hospital is indispensable to promote them. Staffs of DCTSM engage in the risk management at the Research Hospital. Medical accidents and incidents are reported to DCTSM by written forms. When the urgent action is required, the meeting (Iryoujiko Kinkyu Taisaku Kaigi) is immediately held to discuss the first lines of action to protect the involved patient. This meeting also determines the preventive measures. This kind of meeting was held for 23 cases in this year. Medical accidents and the responses of DCTM are reported in the Council of Risk Management in the Research Hospital, which is held monthly.

Educational seminars on risk management are required by regulations to avoid the medical accident. DCTSM took place two seminars and one lecture meeting this year. Through these educations, consciousness for risk management will be tightened. Although medical accidents were reported, no serious events with prolonged /irriversible influences were seen this year, and no suit had seen.

Advise/Review of clinical study protocols before the discussion at the Institutional Review Board (IRB: Chiken-Sinsa-linkai)

Fumitaka Nagamura

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

From January 2004 to December 2004, we received seven protocols and numerous questions

within the research hospital. All the protocols were either Phase I or Phase I/IIa studies. Pre-review of these protocols were finished within two to three weeks from the receipt. The format of pre-review is based on the style of applied in the U.S. Food and Drug Administration. Our opinions are summarized into three sections: safety issue (most concern); major problem; and minor problems/suggestions. These opinions are not obligations which posses enforcement, but those to improve clinical studies. Final decision should be made at the Institutional Review Boards. Furthermore, we performed these activities for other institutes. We received requirements from four institutes.

To assist the planning of clinical studies and writing protocols, we have disclosed "Guideline". Recently many regulations and guidelines were announced. To clear these and to match the Institute's organization, we have been engaging in the revisions of the rules of out institute and in reconstitution of the organization through Working Group.

The role of the translational research coordinator and trials to maintain medical ethics in translational research: Focus on activities related to informed consent.

Momoyo Ohki, Naoko Fukuda, Hajime Kotaki, Fumitaka Nagamura

Translational research is the development of new treatments for currently incurable illnesses, based on cutting edge findings in scientific research. The main purpose of translational research for cancer is the evaluation of safety and pharmacokinetics of current phase I trials. In order to conduct translational research and to maintain the highest medical ethics and scientific methods, Translational Research Coordinators (TRCs) at the Research Hospital of the Institute of Medical Science of the University of Tokyo objectively monitor intra-institutional research from the third party perspective.

TRCs comprise pharmacists, research nurses, certified clinical pharmachologists, registered dieticians, and medical technologists. TRCs monitor and oversee ethical and scientific compliance in their specialized filed. Activities related to informed consent are as follows: 1) indicate inappropriate descriptions in informed consent documents, and propose alternative descriptions to physicians; 2) arrange schedules for the interview of informed consent; 3) provide physicians for exercises on the explanation of informed consent; 4) explain the consent of protocols to participants before informed consent is obtained; 5) when informed consent is obtained, confirm and evaluate the adequacy of explanations given by physicians, and check the understanding and mental condition of participants.

The role of TRC is nearly established, however, such a careful monitoring system will be necessary in the future to achieve a higher level of medical ethics.

Education program for Translational Research Coordinator

Fumitaka Nagamura, Hajime Kotaki and Naohide Yamashita

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 TRCs, we take place seminars after the weekly TRC meeting. To meet the missions of TRC, we prepared handouts by ourselves. The contents of them are the following: what is TR; what is clinical studies; concepts of cancer therapies; concepts of cytotoxic drugs; what is immunotherapy; diseases TRs treat, and so on. Seminars based on these textbooks will be planned regularly. To collect information and to keep up with the development of medicine and environment of clinical studies, we attended the educational programs and lectures. These results were reflected into the education for TRCs. We try to settle the education system for TRC. After the completion of this plan, IMS/UT will be the base for education as well as the one for conducting translational research.

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Research Hospital

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

Professor	Aikichi Iwamoto M.D., D.M.Sc.		教　授(併)	医学博士	岩　本　愛　吉
Lecturer	Toru Iseki M.D., D.M.Sc.		講　師	医学博士	井　関　　徹
Lecturer	Tokiko Nagamura-Inoue M.D., D.M.Sc.		講　師	医学博士	長村(井上)登紀子

Our department manages the transfusion medicine in the hospital including transfusion related examinations. From 2004, transfusion ordering IT system is established for the protection of transfusion accident. In addition, this department is responsible for the supportive function of hematopoietic stem cell transplantation including bone marrow, peripheral blood and cord blood and also various immunotherapy and gene therapy as advanced medicine. The cell processing is an important part of translational research (TR) developed from retrospective clinical study, genome analysis and molecular research. This TR involving human cells has been developing the domestic and international regulations. The projects in our department include 1) Analysis of immune reconstitution post stem cell transplantation (especially cord blood transplantation), 2) Expansion and functional analysis of NK, NKT and T cells for graft-versus-leukemia (GVL) effect and 3) Co-culture system of T cell and mesenchymal cells to expand regulatory T cells for prevention of GVHD, 4) Efficient cell collections for allogeneic- and autologous PBSCT. In addition, recently we started the regenerative study to assess the differentiation ability of the mesenchymal cells derived from long-term cryopreserved bone marrow collaborated with Division of Cell Processing. For the purpose to support these advanced projects, Room for Clinical Cellular Technology (RCCT) has been available for the cell processing. In 2004, RCCT projects include 1) Cord blood cell processing, 2) Thawed cord blood cell washing, 3) Regenerative therapy of osteoblastic cell derived from bone marrow mesenchymal cells (by Division of Stem Cell Engineering (Tooth regeneration)) and 4) Dendritic cell therapy for HIV patient in P3 room (by Division of Infectious Diseases). Our department is the relay point to implement these advanced medicine.

1. Analysis of immune reconstitution post cord blood transplantation

Tokiko Nagamura-Inoue, Satoshi Takahashi[1], Toru Iseki, Arinobu Tojo[1], Tsuneo A. Takahashi[2], Aikichi Iwamoto[3]: [1]Department of Hematology/Ocology, [2]Division of Cell Processing, [3]Division of Infectious Diseases, Advanced Clinical Research Centre, The Institute of Medical Science, The University of Tokyo

Immune reconstitution following unrelated cord blood transplantation (UCBT) in adult patients is of great concern because of immaturity of cord blood immunological cells. We analyzed the twenty-six adult patients (15 to 58 year-old) with hematological malignancies, who underwent UCBT and sustained engraftment were en-

rolled in this study. Infused number of immunological cells in thawed CB units including T cells (CD3+), B cells (CD19+), NK cells (CD3−CD56+), monocytes (CD14+) and also CD34 + cells was analyzed using bead-contained TRUCOUNT tube (BD, CA). Dead cells after thawing were excluded by gating out with 7AAD dye. Immune reconstitution was analyzed every 30 days by 120 days after CBT. Four-color FACS Caliber and TRUCOUNT tube were utilized to calculate the absolute number of immune cells concentration in blood after UCBT. We put strict volume of 50μl fresh unmanipulated blood in each TRUCOUNT tube. RESULTS: Thawed-transplanted NC $2.3\pm\times10^7$/kg, CD34 was $0.72\pm0.3\times10^5$/kg (4.1×106 total), T cells; $3.1\pm1.6\times10^6$/kg with CD4/8 ratio of 3.2±2.0, B cells; $1.2\pm0.5\times10^6$/kg, NK cells; $1.0\pm0.5\times10^6$/kg and monocytes; $1.6\pm0.6\times10^6$/kg. There were no correlations between infused CD34+ cells number and T, B, NK and monocytes numbers. Monocytes increased in blood rapidly after CBT at 30 days, then, declined to the normal value. NK cells was recovered in the early after CBT and then did not so change in number from 30 to 120 days after CBT, while T cells increased time dependent manner, and B cells appeared late but influenced by acute GVHD grade. Within 120 days after CBT, T cells showed also CD4+dominant in most cases with relatively high CD25+CD4+ regulatory T (rT) cells compared to normal control. The patients with grade II to IV aGVHD showed significantly higher number of rT cells on 30 days ($P<0.05$) compared to those with grade 0-I aGVHD. On day 30, the number of rT cells showed 7.7±5.9/μl in grade 0-I aGVHD and 19.4±13.3/μl in grade II-IV. The patients with grade II to IV aGVHD showed significant delayed recovery of B cells on 90 days after CBT compared to those with 0-I aGVHD ($P<0.001$). Conclusively aGVHD in adult patients may influence on the number of regulatory T cells in the early period after UCBT and delayed recovery of B cells

2. Expansion of NK, NKT and T cells and analysis of GVL effect and Co-culture system of T cell and mesenchymal cells to expand regulatory T cells for prevention of GVHD

Tokiko Nagamura-Inoue, Shin Nakayama[1] Kazuo Ogami, Arinobu Tojo[1], Tsuneo A. Takahashi[2], Aikichi Iwamoto[3]: [1]Department of Hematology/Oncology, [2]Division of Cell Processing, [3]Division of Infectious Diseases, Advanced Clinical Research Centre, The Institute of Medical Science, The University of Tokyo

Cord blood transplantation (CBT) is rapidly increasing in number. The characteristics of immune cells of cord blood present CD4 dominant naïve T cells with reduced cytokine secretion, no cytotoxic activity of NK cells, although the cytokine stimulation induced the same level of cytotoxicity as the peripheral adult NK cells. In most CBTs, HLA mismatched cord blood is useful for the engraftment and tolerable GVHD. Unlike cytotoxic T cell, NK cells and NKT cells are known as non-HLA restricted, non-tumor specific cytotoxic activity. Expansion and activation of NK cells and NKT cells might greatly contribute to GVL/T effects and engraftment after stem cell transplantation. We have studied the effect of IL-15 and Flt3L on the expansion and activation of NK and T cell. These expanded cells expressed perforin molecules, and cytotoxic activity against K562 was also recognized, which was inhibited by perforin inhibitor. We further now studied the expansion of T cells with anti-CD3 antibodies and IL15 to induce the GVL effect cells. On the other hand, for the prevention of GVHD, we are now explore the CD 25+CD4+T(rT) on the messenchymal cells derived from the recipient with the after the recovery in CBT.

3. Room for Clinical Cellular Technology (RCCT)

Tokiko Nagamura-Inoue, Kazuo Ogami, Yuka Wada, Kyoko Hirata, Tsuneo A. Takahashi[1], Aikichi Iwamoto[2] and RCCT Steering Committee: [1]Division of Cell Processing, [2]Division of Infectious Diseases, Advanced Clinical Research Centre, The Institute of Medical Science, The University of Tokyo.

Cell therapy including stem cell transplantation and gene therapy for incurable diseases, is urgently needed. It is also mandatory to separate and manipulate cells under quality-controlled sterilized circumstances that could meet with GMP approvals, and provide powerfully enegineered cells to clinical settings. For this purpose, the center having clean rooms (Room for Clinical Cellular Technology: RCCT) with clinical clean room and P3 facilities is now operating in IMS UT. Cell processing of cord blood cells for cord blood transplantation, the dendritic cell therapy for the discontinuance of HAAT therapy in HIV patients are being in execution. Recently, regenerative medicine to induce the osteoblast-like cells derived from bone marrow messenchymal cells is now about to begin.

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Research Hospital

Surgical Center
手術部

Associate Professor Masakazu Hayashida, M.D., Ph.D.
Cliniacal Associate Takahiro Fujimoto, M.D., Ph.D.

助教授 医学博士 林 田 眞 和
助 手 医学博士 藤 本 幸 弘

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

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

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

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

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/analgesics. 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 mechanisms underlying the pain. We also reviewed usefulness of epiduroscopy in pain management in patients with chronic intractable low back and leg pain.

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

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